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Acorus calamus Linn : An important medicinal plant and source of a potent biopesticide

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Abstract

Acorus calamus is an important plant having potential as a resource of medicinal and insecticidal compounds. In Ayurvedic system of medicine, the rhizomes are prescribed antispasmodic, carminative and anthelmintic properties and are given against chronic diarrhoea, bronchial catarrh, glandular and abdominal tumours, intermittent fevers It is also used for treatment of a host of diseases such as epilepsy and other mental ailments. Asarone, a constituent of the oil has been found to possess potent pharmacological action and is a mild sedative, a potent tranquilizer The present review briefly describes the habit and habitat, cultivation, medicinal uses, extraction, physico-chemical properties, economics of production, anti-microbial and insecticidal properties of the oil of *A calamus*

(**Keywords** · medicinal properties/cultivation/anti-microbial/insecticidal/antifeedant activity)

Habit :

Acorus calamus commonly known as sweetflag is semi-aquatic, perennial aromatic herb of the family Araceae. It grows wild and also cultivated abundantly

through out India. Rhizomes are branched, creeping underground, horizontal, jointed, somewhat vertically compressed (5 to 15 cm in length and 1.2-2.5 cm in thickness). They are covered with thin brownish epidermis bearing deep longitudinal wrinkles. The upper surface is covered with triangular leaf scars that encircles the rhizomes springing from each side alternatively. The lower surface bears an irregular zigzag line of small raised root scars[1]. The leaves are grass like or sword shaped, long and slender. The flowers are small, yellow green and arranged in a spadix inflorescence. The fruits are berries and green in color. The seeds are oblong in shape and 1 to 3 numbers in each fruit.

Distribution :

The genus has wide distribution. It is found growing all over the swampy areas, rivers, lakes etc. of the temperate zones in Eurpoe, Asia, America and India. The genus is represented by *A. calamus* in Europe and *A. cochinnensis* in Japan. *A. triqueter* occurs in North-East region of Asia and Siberia. *A. americana* is considered to be indigeneous to America[2]. In India, *Acorus calamus* and *A. gramineus* have been reported to occur. The sweetflags are found growing wild throughout India and Sri Lanka, ascending to 1800 m in Himalayas. It is plentiful in the marshy tracts of Kashmir and Sirmoor in Manipur and Naga Hills[3]. It is found wild or cultivated through out Burma, ascending to 1800 m in Chin Hills[4].

The principle producers of rhizomes of *A. calamus* are Poland, Yugoslavia, Bulgaria, USSR, India, Holland, USA, Japan, East Prussia, Germany and England[5-7]. The Principle centers of exploitation of rhizomes in Yugoslavia are along the Danube, Tissa, Sava and other rivers.[8]

Genetics :

A. calamus is a polymorphic species and exists in different genetic forms. The Jammu variety has shown 36 chromosomes and plants grown in Kashmir have 54 chromosomes. The lowest chromosome number reported for *A. calamus* is 2n=18 by Matsuura and Sato[9]. Cytological investigation carried out by Janaki Ammal *et al*[10] have shown that the Jammu variety is a tetraploid 2n=36=4x and Srinagar variety is a hexaploid 2n=54=6x. This is also the highest chromosomal number reported for the genus. The tetraploid race has been shown to have higher oil and asarone content[11]. Thus the tetraploid plant in *Acorus* posses the maximum capacity for the development of oil.

Cultivation :

A. calamus grows wild in its natural habitats. It is mostly cultivated in Koratage district, bout 25 km from Tumkur, Karnataka state.[12] In recent years, due to renewed interest in the herbal medicines, these plants are cultivated in many regions of India. *A. calamus* is perennial in nature, but still treated as an annual. Planting and harvesting is done year after year. It grows on a variety of soils. The plant is gnerally grown in clayey loams and light alluvial soils of river banks. The field is irrigated and ploughed with green manure before planting. The growing ends or tops of the previous year's crop are planted 30 cm apart, leaving the leafy portions well above the ground. After planting, the field is allowed to go dry for about 10-15 days during which new roots emerge. Thereafter the field is irrigated continuously and drying of the field is avoided while the crop is growing. Dry conditions effect the length and thickness of main rhizome and instead several side shoots or suckers come up. The sweetflag crop needs plenty of water as rainfall or from irrigation. In fact it grows better in standing water. The crop is generally free from pests and diseases apart from stray attacks of leaf spot and mosaic streak diseases.[13] It does not stand extremes of temperature, 10-32°C seems to be the optimum range. The crop suffers with the rise in summer temperature. To avoid this, it is usually planted in March and harvested in January and February. However, it can also be planted all round the year.

The crop is ready for harvest in about a year. The crop maturity is indicated by the falling and yellowing of leaf tips. The plants are dug out, rhizomes removed and the tops kept for the next planting. The rhizomes are washed thoroughly and dried in the sun. The dried material is put into rough gunny bags and rubbed to remove the leafy scales. Rhizomes can be stored in a dry place in gunny bags for 2 years without much deterioration in quality. The tops can either be planted at once or kept covered with dry leaves, straw for 15 days without any deterioration. They could be kept much longer in well moistened open pits. About 80,000 tops are required to plant one hectare. Tops from 1 hectare of *A. calamus* field provide planting material for 2 to 2 ½ hectares.

The increasing demand for sweetflag products has necessitated wider cultivation. In order to meet the demand for the supply of uniform stocking material, several micropropagation protocols for *A. calamus*, using different kinds of explants have been reported[14-17].

Economics :

Yield of the crop varies considerably. The yield of the rhizomes is in the range of 3,750 kg/h and with proper cultivation, almost double the yield is possible. Experimental work carried out at CIMPD Regional Centre, Bangalore has shown that one year old crop yield 13 tonnes of dry rhizomes/hectares, worth Rs. 30,000 (as gross income) @ 2,300/ tonne. The cost of cultivation amounts to about 6,000/h. This results in a net income of Rs. 24,000/h. If there is a demand for its oil, a gross income of Rs. 84,000 could be expected per hactare (Rs. 300 per kg oil). Deducting Rs. 42,000 as distillation charges (Rs 150 per kg of oil) and Rs. 6,000 as cultivation expenses, a net income of Rs. 36,000/h is possible.

Uses in folk medicine :

In India, it is a common practice to administer the dried rhizome, mixed in honey, to babies as a tonic. Powdered rhizome is made into influsion in water and used to wash new born calves as a protection against vermin[18]. Rhizomes are also used for headache, cough, asthma and strengthening of teeth[19]. Dried peeled root is eaten in small quantities as a mild carminative. Dried root is a powerful tonic and is extremely useful in the form of infusion and powder in loss of appetite. Rhizomes are also used as aphordisiac[20]. It is also used as an antidote to croton poisoning[21]. In Southern India, sweetflag is a stock remedy for children's ailments.[22]

The dried rhizome constitute the drug calamus of commerce. In Ayurvedic system of medicine, the rhizomes are prescribed anti-spasmodic, carminative and anthelmintic properties and are given against chronic diarrhoea, dysentery, vertilago, bronchial catarrh, a glandular and abdominal tumours, intermittent fevers, flatulence and other disorders associated with indigestion[23-26]. It is also used for treatment of a host of diseases such as epilepsy and other mental ailments[27-28]. They are also employed for kidney and liver troubles, rheumatism and eczema. The roots possess antifertility properties. Sweetflag oil was found to possess anti-ulcer and cryoprotective properties[29]. The alcoholic extract of *A. calamus* roots and rhizomes possess sedative and analogesic properties and cause a moderate depression in the blood pressure and respiration. Asarone, a constituent of the oil has been found to possess potent pharmacological action and is a mild sedative, a potent tranquilizer, mild hypotensive and hypothermic substance[30]. When administered intraperitonially it behaves like a CNS depressant, producing no side effect[31]. α-asarone and β-asarone enhances the anesthetic activity of pentobarbitone and hexobarbitone. Hypotonic potentiating and also tranquilzin activity of β-asarone is significantly higher than that

of asarone. These cause a fall in rectal temperature, show anti convulsant activities, decrease in sociability score and an anti-cholnergic action. Elimination of phenolic and aldehydic frctions from the oil results in the incrẹase in toxicity andsedative potentiating activity. The oil is used in the preparation of aromatic cordials and liquors for flavouring beer and also perfumes[32]. The oil of calamus has been used as a flavouring agent in food and drug preparation. But β-asarone has proved to be carcinogenic in several animal tests. For safety reasons the use of β-asarone rich races should be avoided.

Calamus oil :

The rhizomes, roots and leaves yield a light brownish yellow volatile oil known as calamus oil. The oil is viscous liquid, warm, woody and spicy and has pleasant odour with sweetness. The oil yield the *A. calamus* depends on the period of growth of the rhizomes, location, seasons of collection and temperature[34-35] (Table-1). The yield of oil from different parts of plant is as follows; fresh rhizomes up to 1.8, dried rhizomes 1.5-3.5, leaves 0.2 and fresh aerial parts 0.12%. The highest yield of oil is obtained from dried, unpeeled rhizomes. Wulff[11] reported that the amount of essential oil in the

Table 1– Variation in oil quantity of *A calalmus*

Sl. No.	Collection of plant material	Source of plant material	Oil content (%)
1.	Region	Coimbatore	2.5
		Jammu	3.1
		Kashmir	1.4
		Bangalore	2.8
2.	Plant parts	Rhizomes (fresh)	1.8
		Rhizomes (dried)	2.5
		Leaves	0.2
		Aerial parts	0.12
3.	Chromosomal number	Diploids	2.17
		Triploids	3.12
		Tetraploids	6.82

dried rhizomes of *A. calamus* increased with the chromosomal number. Diploids have an average content of 2.17%, 3n plants 3.12% and 4n plants 6.82%. The unpeeled dried European calamus roots yield 1.5 to 4.5% oil, whereas the Japanese material yield up to 5% oil.

The important constituents of Indian calamus oil are asarone (up to 82%) and its β-isomer[36-37]. The other constituents are calamen 0.1-0.5%; calmene-0.4%, calamenone-1.0%; methyl eugenol –1.0%; eugenol- 0.3%; -pinene and camphene-0.2%. Presence of small quantities of palmitic, heptylic and butyric acid, asaronaldehyde, calamol, calmone and azulene has been reported. Sesquiterpenic ketones like acorene, calrene, calacone, calacorene, acorenone, acolamone, isoacolamone, epishyobunone, shybunone, isoshyobune and acoragermacrone and alcohols like isocalamendiol and preisocalmendiol are also present. The hydrocarbons present in the oil are elemene, caryophyllene, calomeneone, calamenene, cadalene and humulene[38-46].

The physicochemical constants of the oil of the rhizome obtained from different places are presented in the Table 2. Great variation in quality of the calamus oil was observed in plants collected from different geographical areas[47]. Indian oil is not identical with that from Eurpoean varieties and it resembles the Java Oil[48]. Investigations carried out by Choudhary *et al*[49] revealed that oil obtained from calamus roots from the Kashmir valley was different from the oil obtained from the Jammu area. However, the Kashmiri oil resembles the European oil in the properties and composition. The Indian oil contains 82% of asarone while the other commercial varieties have only about 7% of it. The asarone content is more in tetraploids, calamine and camphor contents are more in the hexaploids. Wulff[11] reported that the essential oils from diploid races contained large proportions of geranly acetate and asarones, whereas these are practically absent in the triploids. The tetraploid race contains less asarone than the triploids in the leaves, but more in the rhizomes. Predominating constituent in the oil from Jammu area is asarone (90%) whereas in the Kashmiri oil, hydrocarbons are more and asarone is found to be only 5.2%. Japanese rhizomes are less popular for the production of flavours and perfume oil. The Indian oil differs from the Europian oil by higher specific gravity and lower optical rotation, higher refractive index and better solubility. Polish and Yugoslavian oils are often pale colored and display a delightful sweet, uniform lasting odour and are best from perfumery point of view. The variation in the quality of calamus oil is often due to the botanical starting material. Dried roots produce more oil than the fresh rhizomes. From perfumery point of view, the oils from fresh roots, particularly those of Poland and Yugoslavia origin are the best.

Table 2–Physico-chemical characters of calamus oil from different sources.

Source/ Place	Yield (%)	Specific Gravity	Refractive Index	Optical Rotation	Acid value	Easter Value	Saponification value
Bangalore India	2.8	1 076	1.5461 (at 30°)	-1°50'	2 4	.	4 1
Jammu India	3.1	1 056	1 5540	+2° (at 15°)	0 14	14	..
Kashmir India	1 4	0 971	1 5036	+14°	2 24	12 7	.
Europe	0.94 –2 2	0 960 –0 974	1 5045 – 1.5070	+15°50 to +18°40'	Up to 3 7		6.8 –7.5
America	3 3	0.950 – 0 974	1 5013 –1 5069 (at 20°)	13°48' + 15°	.	.	8 4 –10.7
Japan	4.63	0.975 – 0.985	1 5051–1.5090 (at 20°)	+2°80' to 13°40'	. .	.	. .
Russia	3.58-7.8	0.936 – .955	1.5029 – 1 5130		1 6 – 3 9	7 6 –13 9	5 8 – 10.7
Yugoslavia	1.3–4 1	0.960 – 0.971 (20°)	1.50 (at 20°)	-13° to -27° (at 20°)	1-2	7-15	. .

Anti-microbial properties :

The applications of extracts of higher plants for the control of plant diseases were reported from long past. The oil showed marked anti-tubercular action *in vitro* and inhibited the growth of *Mycobacterium tuberculosis* at a concentration of 10 mg/ml.[50-52] The oil inhibits the growth of gram negative organism at concentrations ranging from 0.4 to 0.6 mg/1. It has no inhibitory effect on the growth of gram positive organisms. However, it inhibited the growth of *Diplococcus pueumonia* at a concentration of 0.6 mg/1. The antibacterial activity of *A. calamus* was reported aganist some pathogenic and non pathogenic bacteria[53-54]. The alcoholic extract inhibited the growth of certain fungi[55-57]. In our laboratory, we reported the antibacterial action of *A. calamus* against certain bacteria[58].

Insecticidal and pesticidal properties :

The uses of plant products as insecticides and pesticides are known for many years and number of such compounds have been isolated from various plants[59-60]. The toxicity and insecticidal activity of *Acorus* is well documented in the literature (Table 3). Traditionally, powdered rhizome of *Acorus* is used as insecticide for the destruction of the fleas, bedbugs, moths, lice etc. It is effective in killing insect pests in stored rice and is considered to be better than chemical pesticides as it shows no residual effect. Subramanyam *et al*[61] has shown the direct use of powdered rhizome and found to be useful against the bedbugs, moths, lice *etc.* Mukerge and Govinda[62] found that ether, petroleum ether and alcoholic extracts of *A. calamus* possess insecticidal activity. The ether extract of the rhizome exhibits ovicidal and insecticidal properties with no residual toxicity. Dixit *et al*[63] studied the insecticidal activity of solvent extract and steam volvatile principles of rhizome of *A. calamus* against the housefly (*Musca nebul)*, mosquito (*Culex fatigans*) and the furniture carpet beetle (*Antrenus vorax*). They reported that the solvent extract and the steam volatile principles of the rhizome are toxic to house flies and mosquitoes and exhibit synergistic activity when used in combinations with the DDT against flies. Mirnovo *et al*[64] reported that adult *Anopheles meculipennis* and *Musca domestica* adults are killed within 40 minutes when sprayed with decoction of *A. calamus* rhizomes. The toxicity of *Acorus* against *Musca domestica* was reported by Singh and Singh[65]. Inability of egg hatching of *Drysdercus koeinigi* was recorded at 100 ppm concentration of *Acorus oil*[66]. Saxena *et al*[67] in their study, mixed the grains with the crushed segments of rhizomes, in closed containers to control the insects like *Callasobruchus chinensis, Sttophilus orizae, Corcyra cephalonica, Trogoderma granarium.* In another experiment, when the

pieces of rhizome or its oil were placed at the bottom of the containers, stored grain pests were controlled effectively. Experiments conducted at Central Rice Research Institute, Cuttack, showed that the powder of the rhizome of *A. calamus* has an excellent insecticidal properties. The powder was used at the institute to control the insect pests of stored paddy. The rice treated with sweetflag and stored for a year, did not show any unpleasant odour when cooked[68]. Emulsion of essential oil of *A.*

Table 3–Insecticidal activity of various parts of *Acorus calalmus*

Sl No	Properties	Nature of the compound	Reported activity against different pests
1.	Insecticidal propertis	Powedered rhizomes Pet ether extract solvent extract	Bed bugs, moths, lice Housefly, mosquitoes and many insect pests
2.	Toxic	Acorus oil Powder of Acorus Acorus oil β-asarone Acorus oil	Housefly Melon fly *Prostrephanus truncatus* *Prostrephanus truncatus* Potato beetle, *Callosobruchus phaseoli,* *Sitophilus granarius*
3	Antifeedants	Acorus oil Oil Synthetic derivatives of Acorus	*Peridroma saucia* *Spodoptera litura* Stored grain pests
4	Antigonadal	Acorus oil	*Dyosderus koenigii* *Sitophilus zea mais* *Aphis carcivora* *Oncopeltus fasciatus* *Prostrephanus truncatus* Many stored grain pests

calamus showed an excellent knock down effect on the beetles and posses a long lasting residual effects[69].

Antigonadal agent :

This concept is not extensively discussed in literature though few reports are available regarding the inhibition of oviposition by certain terpenoids[70]. Comprehensive studies on *A calamus* have revealed that asarone, the main component of extract is known to be chemostrerilant,[71-79] toxic[80-91] morphogenetic agent,[92-94] and ovioposition stimulant[95] to the insects. It produces specific effect on gonands in which terminal oocyte is the first target in treated females. Vapours of oil are reported to have sterilizing effect on the ovary of *Dyosderus koeinigi* and *Trogodrma granarium* Everts[96]. The vapours of the oil when blown over a number of stored grain pests exhibited a complete inhibition in their ovarian development.[97] The inhibition in the gonads is due to blocked intestitial cell secretions.[98] Same activity is reported in male insects showing sperm malformation and agglutination.[99] The compound caused resorption in many insects leading ultimately to complete regression of the ovaries. To find which of the substituent groups in the aromatic ring on the side chain of β-asarone was specifically inhibitory, trials were conducted by Saxena *et al.*[100] In their study they found that position of different groups in the aromatic ring is known to play an important part in various activities, whereas the position of the hydroxyl group is responsible for activities such as anti-assimilation and reduction of pupation. O-methyl group in the ring and double bond in the side chain of β-asarone plays an important role in the antigonadal activity. Comparison of activities of compounds suggested that the cis configuration of side chain double bound in β-asarone is important for antigonadal activity. This type of activity has given rise to the new concept towards pest management.

Antifeedants :

Though a number of plant products are being investigated as antifeedants, studies on the role of essential oils for this activity are still scantly[101]. A number of diterpenoids and triterpinoids have been found to possess feeding inhibitory activity. The feeding deterrence activity of *Acorus* was reported against many pests[102-104]. Recently Koul[105] and Sharma *et al*[106] have reported antifeedant properties of essential oils of *Acorus* against *Spodoptera litura.* In our laboratory, we reported the feeding deterrence activity of rhizome extract of *Acorus* against *Spodotptera*[107]. Data from the literature shows that both asarones (α and β asarone) are growth inhibitors and antifeedants against the larvae of the *Peridroma saucia* Hubner[108]. Synthetic and

derivatives of asarone is also reported to posses antifeedant activity against stored grain pests. However these isomers have different mode of action i.e. β-asarone is toxic whereas α-isomers acts as antifeedant with no toxicity. In order to see which is having high deterrence and at the same time safe as food, 5 isomers of α asarone were synthesized chemically and tested against three stored grain pests[109] i.e. *Sitophylus granaries, Tribolium confusum, Trigoderma granarium.* All synthesized isomers showed antifeeding properties against stored insect pests. This may be due to the α and β asarone, which are substituted alkyle benzenes may exhibits high reactivity with respect to selected nutrophilic factors which occur in proteins and cells. In asarones, there exists a highly reactive benzyle moiety which may interact with SH thiole groups. These can form active alkylating agents which can form stable combinations with SH thiole groups of endocrine glands and also with intestinal cells of the insects and thus exerts their deterrence activity against storage pests[110].

It can be concluded from the studies reviewed in the present article that *A. calamus* is an important medicinal plant, widely used in Ayurvedic system of medicine to treat many diseases. It can be grown in many geographical regions with varying climatic conditions. The rhizome extract yields calamus oil, mainly used in perfumery and flavouring industry. The oil also possess anti-bacterial and antifungal properties. The rhizome extract and essential oils have varying degrees of pest controlling activity in the form of insecticidal, antigonadal and antifeedants. Antigonadal properties of this plant is worth mentioning, as it offered a new and safe approach towards insect control.

In keeping view of above important characters, an effort has to made for large scale cultivation of *A. calamus* in different regions of India. Focus should be shifted toward the isolation and identification of active principles of essential oil by using advanced technology. Studies should be conducted to asses the phytotoxic effect of oil against the major agricultural pests and their evaluation at field level.

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Biology and food utilisation efficiency of *Eurema hecabe* (Lepidoptera : Rhopalocera : Pieridae)

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Abstract

The study established that the pierid butterfly *Eurema hecabe* would be on wings and breed throughout the year, with September-November as the most favourable period The polyphagous larvae utilise several legumes as hosts including *Cassia tora* on which the larvae were reared and studied. At about 28°C of laboratory temperature, the life history was completed in 20-27 days, with eggs lasting 3-4, larvae 11-16 and pupae 6-7 days. The short life history permitted 11-12 broods yearly, making the butterfly multivoltine. Among the five instars obtained, the last two accounted for 73.7% of total weight gain for a food consumption of 66.4 % of the entire larval period. The values of CI, GR and AD across the instars decreased as the larvae aged The average values of CI and GR are 0 59 and 7 71 respectively, and that of AD is 91 4 %. In contrast the values of both ECD and ECI increased as the larvae aged; their respective average values are 14 02% and 11.96%. The adult foraged on 19 floral species, with nectar sugar concentration varying from 16-61% and, mostly received pollen on proboscis and head, thus conforming to psychophily.

(**Keywords** . *Eurema hecabe*/life history/food consumption and utilisation/nectar resources/ psychophily)

Introduction

Over 1500 butterfly species occur in India. Their populations have gone down in the past four decades[1] and several of the species reached the threatened category[2]. They need to be protected, improved and managed effectively for ecological and environmental reasons, especially for the key role they play in natural pollination[3,4,5,6] To achieve any success in this direction a complete knowledge of life history, seasonality, larval and adult food requirements of butterflies is essential[3]. For most Indian butterflies, such information is largely inadequate[4,5], but is necessary for rearing the species and releasing the adults into the wild, a strategy often suggested to restock the depleting population as part of a conservation measure. Here the related information is provided for the common grass yellow butterfly *Eurema hecabe* (Moore) of Pieridae. This small fragile butterfly is one of the six species of *Eurema* (=*Terias*) distributed in India and described as abundant throughout the plains in

earlier writings[7]. But it is not found in abundance nowadays, perhaps due to urban and agricultural expansions resulting in the destruction of natural areas. This work deals with adult nectar resource, life history, larval food consumption and utilisation, egg, larval and pupal development success and seasonality for use in the conservation management of this butterfly species.

Materials and Methods

During the major study on the biology and ecology of South Indian butterflies, the common grass yellow butterfly *Eurema hecabe* (Moore) of Pieridae was found ovipositing on several leguminous plants including *Cassia tora,* C. *occidentalis,* C. *siamea, Mimosa pudica, Samanea saman* and *Peltophorum.* The eggs laid on *Cassia tora* were used for the present study. The host plants *Cassia tora* distributed at the Indira Gandhi Zoological Park at Visakhapatnam (17° 42' N 82°18'E) were observed for enumerating the eggs, larvae and pupae once every month. Freshly laid eggs along with the substrate leaf material were collected in Petri dishes of 4 and 9 cm diameter and brought to the laboratory at the Andhra University and incubated at around 28°C. The emerged larvae were maintained on tender fresh leaves of C. *tora* and were followed until pupation. The number of instars as indicated by skin shedding were noted. Particulars of each instar including food consumption and weight gain were recorded. Using the formulae of Waldbauer, larval performance was assessed in terms of Growth rate (GR), Consumption index (CI), Approximate digestibility (AD), Efficiency of conversion of ingested food to body tissue (ECI), and Efficiency of conversion of digested food into body tissue (ECD). The relation of food consumption and growth of larva was analysed statistically using Karl Pearson's regression and correlation. Prepupal behaviour and pupal characters were recorded. Adult nectar resources, their blooming periods, corolla lengths, particulars of nectar, butterfly proboscis length, foraging speed and diurnal activity were recorded.

Results

Adult description and nectar resource

Adult wingspan is 20-25 mm. The male is bright yellow with upper forewing apex and termen broadly black, and upper hindwing with narrow black terminal border. The female also bears broader black border on the forewings. The underside of forewing of both male and female bears two black spots. Both male and female individuals feed on floral nectar. About 19 different plant species have been found to be utilised as nectar resource (Table 1). Their flowers with corolla lengths of 3-22 (av. 6.1) mm are conveniently handled by E. *hrecabe* with its 20 mm long proboscis. Nectar volumes varied from 0.01-0.9 µl, and sugar concentration from 16-61 % with

glucose dominance in 11 of the 14 species examined. The number of flowers visited per minute varied from 1-17 (av. 8), and the time spent at a flower from 1.6-15.0 (av.6.4) seconds (Table 2). Pollen pick-up by different body parts of *E. hecabe* was not uniform with the 18 plant species examined. Pollen of 16 species got deposited on proboscis, 6 species on head and legs each and 3 species on antennae. Contacts with the stigma varied similarly. Foraging activity took place during 0700-1800 h on fine weather days, and the period of higher activity varied from floral species to species probably depending on the times of anthesis (Table 3).

Table 1–Nectar sources of *Eurema hecabe.*

Name of plant species	Flowering period
Antigonon leptopus	Year-long
Borreria hispida	Jul - Oct
Duranta repens	Jun - Dec
Eupatorium triplinerve	Year-long
E majus	Nov - Jan
Helianthus debilis	Year-long
Hyptis suaveolens	Sep - Nov
Lantana camara	Year-long
Merremia tridentata	Aug - Oct
Ocimum basilicum	Jul - Sep
Pedalium murex	May - Aug
Premna latifolia	May - Aug
Sida cardifolia	Aug - Dec
Stachytarpheta indica	Jun - Sep
Tribulus terrestris	Jun - Oct
Tridax procumbens	Year-long
Vitex negundo	Year-long
Zizyphus oenoplia	Aug - Dee
Z. *mauritiana*	Aug - Oct

Life history and seasonality of life stages

The eggs are laid singly, are oval, whitish with smooth surface, and 0.7-1.2 (1.0 ±: 0.02) mm high hatching in 3-4 days. The larvae pass through five instars each of the first four instars continues to grow for 2-3 days before they cast their skin. The fifth instar lasts 3-4 days. Larval growth is continuous and increases from a length of 1.2-2.0 (1.8 ± 0.1) mm in first instar to 22.0-24.5 (23.1 ± 0.11) mm in fifth instar. The cylindrical body is pale creamish initially, but becoming dark green subsequently. As the larva completes the third instar stage, the entire body becomes hairy. The segmented body bears stripes on dorsal surface appearing green with black markings: These characters persist up to the last instar; additional characters at this larval stage include the pale yellow colour and a small black band on the lateral sides of the body. The fully grown fifth instar pupates within one day. The pupa is yellow to pale green colour, 16.0-17.5 (17.0 ± 0.1) mm long with its anterior part drawn into a snout of 4.5-5.4 (5.0 ± 0.02) mm long. The pupal stage lasts 6-7 days. The development success rate of different life stages in different months is high, it being 80-100% for eggs, 83-100% for larvae and 86-100% for pupae (Table 4). Monthwise distribution of eggs, larvae and pupae monitored through searches of these life stages on 15 different plants of *Cassia tora* indicated that the three life stages occur throughout the year in considerable frequency but with a higher density during September-November period (Table 5) and this period corresponds well with the period of their higher development success rate.

Consumption and utilisation of food by larvae

Quantitative data on consumption and utilisation of food were obtained feeding the larvae on *Cassia tora* leaves. Weight of food ingested, weight of faeces, weight gain in larvae and the food consumption, growth and food utilisation indices were calculated for each of the five instars (Table 6). The food consumed by instar II was 8 times more than that of instar I, that by instar III was 5 times more than that of instar II; that by instar IV, was a little more than that of instar III and that consumed by instar V was also slightly more than that of instar IV. Thus, there was tremendous increase in food consumption between instar II and III. The same was reflected in the weight of the larvae of instar III. The rate of intake of food relative to the mean weight of the larva as indicated by the consumption index (CI) was high in instar 1. The rate decreased slightly with instar II and then decreased steeply from instar II to instar III and rather slowly from instar III to instar IV and also from instar IV to instar V. The AD values decreased across the instars. The ECD and ECI values showed an increase through the successive instars. The increase was rapid from instar II to instar III, thereby indicating that the efficiency with which digested food was converted to body substance was more at instar III.

Table 2– Nectar chaucterıstics of the floral species and foraging speed of *Eurema hecabe*

Name of plant species	Nectar volume (µl)		Nectar concentratıon (%)		Sugars	Foraging speed	
	10.00 h	17.00 h	10 00 h	17.00 h		# flowers visited per mın	Time spent (seconds)
Antıgonon leptopus	0 02	0 90	62	59	Gsf		
Borreria hıspıda	0 02	0.02	28	32	Gsf	17	1.6
Duranta repens	0 60	1.00	24	19	gSf	-	-
Eupatorıum trıplınerve	0 80	0 40	34	31	Gsf	1	15 0
Hyptıs suaveolens	0 02	0.04	24	19	Gsf	-	-
Lantana camara	0.06	0.04	18	23	gSf	8	3 2
Ocımum basilıcum	0.01	0 01	18	16	Gsf	-	-
Premna latifolıa	0.01	0 06	36	33	Gsf	-	-
Sida cordıfolıa			Traces of nectar		Gsf	4	3.0
Stachytarpheta indica	0.90	1.00	27	28	gSf	14	3.5
Tridax procumbens	0.50	0.90	29	25	Gsf	2	13.7
Vıtex negundo	0.03	1.00	20	25	Gsf	9	4.8
Zızyphus maurıtıana	0 02	0.02	57	51	Gsf	-	-
Z oenoplıa	0 02	0.02	53	49	Gsf	-	-

Sugars (Glucose, Sucrose, Fructose) · Capital letter ındıcates dominance.

Table 3– Diurnal activity pattern of *Eurema hecabe* on floral species (visits in each hour given as percentage of total daily activity).

Name of plant species	Time of day (h)											
	6-7	7-8	8-9	9 - 10	10 - 11	11 - 12	12 - 13	13 - 14	14 - 15	15 - 16	16 - 17	17 -18
Antigonon leptopus	0	0	15-20	**25-30**	10-15	0	15-20	20-25	0	0	5-10	0
Borreria hispida	0	1-5	25-30	20-25	**30-35**	5-10	0	0	0	0	0	0
Stachytarpheta indica	0	25-30	**30-35**	0	15-20	20-25	0	a0	0	0	0	0
Tridax procumbens	0	0	0	0	0	10-15	15-20	**40-45**	1-5	15-20	0	0
Vitex negundo	0	0	0	5-10	**20-25**	**20-25**	15-20	10-15	5-10	1-5	1-5	1-5

Table 4– Development success of different life stages of *Eurema hecabe* in the laboratory

Life cycle stage	A	S	O	N	D	J	F	M	A	M	J	J
# eggs incubated	15	27	20	25	30	25	10	10	12	8	10	8
# larvae hatched	12	25	18	25	29	24	9	9	11	8	8	7
# pupae formed	10	24	18	25	29	23	9	9	10	7	7	6
# adults emerged	10	24	18	25	29	22	9	9	10	7	6	6

Table 5– Monthwise distribution of different life stages of *Eurema hecabe* on *Cassia tora* in the field

Life cycle stage	J	F	M	A	M	J	J	A	S	O	N	D
# Eggs	14	14	12	11	11	9	12	20	40	38	30	17
# Larvae	4	6	6	2	4	2	4	8	20	15	10	12
# Pupae	2	2	1	1	2	1	1	2	9	8	7	4

Table 6 – Food consumption and utilisation efficiencies of *Eurema hecabe* larvae on *Cassia tora* leaves

Instar number	Weight of food ingested (mg)	Weight of faeces (mg)	Weight gain by larva (mg)	GR	CI	AD %	ECI %	ECD
I	6.0 ± 0.08	0.04 ± 0.02	0.06 ± 0.01	0.80	16.00	99.0	1.0	2 69
II	48.0 ± 0.02	1.20 ± 0.08	2 87 ± 0.06	0.87	14.60	97.5	5.9	6 13
III	232.0 ± *0.24*	12.90 ± 0 09	36 60 ± 0.11	0.81	5.40	94.4	15 7	16.70
IV	251. ± *0.26*	29.10 ± 0.10	37.70 ± *0 11*	0.30	1.64	85 5	18.7	21.80
V	*313 0 ± 0 32*	60.20 ± *0.17*	56.50 ± *0 18*	0.17	0.93	80.7	18.5	22.80

Discussion

Eurema hecabe utilises several legumes as larval hosts and is thus polyphagic in its larval feeding strategy. It did not attain pest status on any of the host plants examined. But a species of *Eurema (E. blanda)* which lays its eggs in large batches, is reported to assume pest status on *Albizzia*[9,10]. All other species of the genus including E. *hecabe* lay eggs singly. The strategy of single egg laying is advantageous in that the breeding females can use many host plants. and different species of the host genus within a habitat[11,2], and this advantage is being enjoyed by *E. hecabe.* Normally, the larvae from such species laying eggs singly are less likely to defoliate their hosts.

The eggs hatched 3-4 days after incubation in the laboratory at around 28°C. The individual larvae had four moults and five instars : over a period of 11-16 days. The pupal period was 6-7 days. Thus, the total period required for the egg to develop into adult is estimated to be 20-27 days. As temperature influences the length of each stage of life history[3,14,15]. the length of each life history stage and thus the total length of life history of *E. hecabe* may vary in other regions of its distribution in the country depending on the prevailing temperatures. Thus, in Bengal the larval period was longer (20 days), and the pupal period ranged between 5-25 days[7]. Another pierid butterfly *Pieris rapae* had the best development of eggs, larvae and pupae at 27°C (Table 3 of Richard[13]). The length of different life stages of *E. hecabe* are comparable with those of *P. rapae,* and the temperature regime favourable for reproduction of these two pierids appears to be similar.

Maxwell-Lefroy[7] writes that *E. hecabe* may have four broods in places of climate extremes, and 12 broods in places of favourable climate. In general adult life span of a butterfly may extend from 7-12 days[16]. If this general period of life expectancy is applied to *E. hecabe* which required 20-27 days for the completion of life history, 11-12 broods may be reasonably expected yearly in the environs of Visakhapatnam enjoying coastal climate without any extremes. The year long distribution of eggs, larvae and pupae on the host plants in field condition supports this estimation of 11-12 broods and also show that September - November period is most conducive for *E. hecabe* reproduction, and this period falls within the northeast monsoon (September - December). This peak distribution agrees with Wynter-Blyth[17] who expressed that the distribution of butterflies at a locality mostly depends on rainfall conditions.

The profiles of food consumption and weight gain of successive instars ran on similar lines, both showing a progressive increase as the larvae aged. The regression of weight gain on food consumption yielded an equation: Y = 0.98 X-98.34, with the value of r = 0.92 exceeding the table value of t = 0.78 (at 1 % level), thus indicating a linear .relationship between the two variables. The weight gain of the last two instars accounted for 73.7% of total weight gain for a food consumption of 66.4% of the entire larval period. This rise in food consumption and growth in the last two instars may be taken as a strategy to compensate for the energy required by the nonfeeding pupal stage, and is reported in several Lepidoptera[8,18,19,14,15,20].

Food consumption index (CI), growth rate (GR), and approximate digestibility (AD) - all behaved in a similar way with the values decreasing as the larvae aged. The average values of CI and GR are 0.59 and 7.71 respectively; the first two instars recorded values higher than these averages. The percentages of AD ranged between 99.0 - 80.7 (av. 91.4), the first three instars registered higher, AD values than the mean. High AD values are often expected when the foliage forms the food rich in nitrogen (and water)[20,21]. Compared to the AD values, those of ECD and ECI are low, the ECD values ranging from 2.69-22.80 (av. 14.02) and of ECI from 1.0 - 18.5 (av. 11.96). Both ECD and ECI showed a similar pattern with the values increasing with the age of the larvae. The relatively high values of the last two instars (ECD = 21.80, 22.80; ECI = 18.70, 18.50) are indicative of the quality of *Cassia tora* as food for E. *hecabe* larvae. Being a legume, C. *tora* foliage must be rich in nitrogen and being an annual forb its leaf water content might be in the range of 75-95%[19,23]

Both larval and adult resources are the basic prerequisites of habitat suitability for butterflies[24,25]. *Eurema hecabe* utilises many legumes as larval and 19 different floral species as adult nectar resources. Floral nectar is a vital source of sugars and amino acids[26], and provides energy for flight, which is vital to find mates and food plants on which to lay eggs[25]. The choice of oviposition host species by the polyphagic *E. hecabe* is likely to depend on adult requirement of floral resource as demonstrated in the choice of oviposition host species in *Euphydryas chalcedona*[27]. Nectar intake might increase adult longevity, egg production and egg maturation[28,29,30]. The range of sugar concentration of floral species of E. *hecabe* (16-61 %) corresponds well with 15-50% of psychophilic floral nectars[31]. While foraging at flowers, E. *hecabe* received pollen mostly on proboscis and head, thus complying with an important requirement of psychophily[31] and proving its effectiveness in pollinating its floral species.

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Bacillus sphaericus resistance in mosquitoes : An approach for resistance management

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Abstract

The last decade has evidenced an increased interest in biological vector control agents for mosquito control operations The discovery of highly potential microorganism like *Bacillus sphaericus (Bs)* to control mosquito larvae has witnessed promising results. However, recent reports on development of resistance to *B sphaericus* by *Culex* species have impeded the progress In the present study, an attempt has been made to evaluate the efficacy of malathion® (OP-compound, 95% purity) against a *B sphaericus* resistant population of *Culex quinquefasciatus* Say that have been reared in the laboratory for more than five years. We have observed that the resistant larvae showed a high level of resistance against *Bs* 2362 strain. The resistance ratio (RR) calculated between *Bs* resistant and susceptible larvae were 1166 9, 1011.99 and 971.7 folds at LC_{50} , LC_{90} and LC_{95} levels respectively. However, we have not observed any significant difference in the susceptibility level between *Bs* resistant and susceptible larval strains (no cross-resistance) to malathion This observation suggest that malathion may be of use for the management of *B sphaericus* resistance in mosquitoes

(**Keywords :** *Bacillus sphaericus*/malathion/*Culex quinquefasciatus*/resistance/cross-resistance/ management)

Introduction

Bacillus sphaericus (*Bs*) is one of the bacterial organisms, which is now used in mosquito vector control operations as bioinsecticide [1, 2]. The toxic activity of bacteria is specific, restricted to mosquito larvae. The activity is directly related to the synthesis of parasporal crystals during sporulation [3]. The parasporal crystal toxin is made of two proteins of 51 and 42 kDa (P51 and P42 respectively) and both components are required for full expression of larvicidal activity [4]. Crystal toxins are ingested by the larvae and after solubilization and proteolytic cleavage, the activated

toxin interacts with the midgut epithelium leading to death of susceptible larvae [5]. The toxin binding mechanism to a specifc receptor sites in midgut brush border membrane (MBBM) of mosquitoes are also elucidated [6].

Though *B. sphaericus* is potential bioinsecticide, the recent appearance of resistance in *Culex* species have impeded the progress[7-10]. So, identification of appropriate methods to manage resistance against *B. sphaericus* in particular to filarial vector of *Culex quinquefasciatus* Say may give basis for development of better methods to prevent or delay resistance problem in treated mosquito population. We undertook this study to evaluate the efficacy of malathion (organophosphate compound) for the management of *B. sphaericus* resistance in *Culex quinquefasciatus* Because, malathion is often used to control various species of mosquito vectors and agricultural pests as it is having low mammalian toxicity and low persistance in the environment compared to organochlorines[11]. So, the use of malathion to manage resistance to *B. sphaericus* to *Culex quinquefasciatus* may be an useful approach in this direction.

Materials and Methods

Mosquito colonies

Third instar larvae of *Culex quinquefasciatus,* susceptible to *B. sphaericus* were used from a colony maintained for more than five years in CRME, Madurai and this strain is named as Madurai susceptible strain, (MS). The eggs and larvae collected from the field (Meenambikainagar, Madurai) were used to establish this colony.

A *B. sphaericus* resistant colony of *Cx. quinquefasciatus,* collected from the field (Gandhinagar, Kochi, Kerala, South India), where resistance has been reported[7] was used in the present study. This resistant colony (named as Gandhinagar resistant strain, GR) has been subjected to selection pressure (thousand late third instar larvae from GR strain were subjected at a concentration of 990 mg / lit in 3 litre capacity bowl to determine the mortality of larvae at moderate level. The mortality ranged from 30 to 40 %. The surviving larvae from this experiment were pooled, rinced in de-ionized water and reared to the next generation. Late third instar larvae of this generation were again subjected to different doses to determine the lethal concentration level, and the survivors were reared to the next generations for more than five years in the laboratory[8].

All mosquito species were reared in the laboratory at ambiant laboratory temperature (29 - 31° C) in enamel trays by feeding the larvae on a ground mixture of yeast and dog biscuit (1: 2). The larval food was sprinkled over the water surface after the scum of left over food from previous day has been removed. Pupae were removed every day from the enamel trays containing larva. The pupae were placed in adult mosquito cages where the emerging adults of both sex were held for oviposition. Females mosquitoes were fed blood meals from live chicken and male mosquitoes were fed with 5% glucose solution through cotton pads and water soaked raisin. Adults were allowed to oviposit in enamel cups with water kept inside the rearing cages. Freshly hatched larvae from egg rafts of two larval strains (GR, MS) were cultured individually as cyclic colonies.

Bioassay

Lyophilized bacterial culture of *B. sphaericus* 2362 (SPH-88) (titre : 1500 International toxic units/mg *Bs* toxin) received from Institute Pasteur, Paris, France, was used as toxin source. Titration and preparation of stock solution from this bacterial sample and bioassay were made as described in WHO protocol[12] In the present study, 19 gms of *Bs* spore/crystal toxin were homogenized in 650 ml of deionized water and stock solution was prepared. The aliquots of appropriate dilutions (two fold dilutions) ranging from 29.23 to 0.46 gm / lit and from 38.97 to 0.61mg/lit were used for *Bs*-resistant and susceptible larval strains respectively. These ranges of toxin were neccessary to determine the susceptibility levels from 1.0% to 100% (in 48 hr) by keeping 7 concentrations. Bioassays were conducted in disposable polythelene cups (200 ml capacity). Test medium was prepared by adding appropriate concentration of *Bs* toxin as the range mentioned above, in 150 ml of deionized water and twenty early third instar GR and MS larval strains were introduced seperately in each of the test concentrations. Larval food was given for *Bs* resistant and susceptible larvae as recommended by WHO. Adequate replicates (two replicates) were placed in every experiment. We have repeated the experiments three times. The experimental larvae were held at a room temperature (~31 °C) and larval mortality was assessed 48 hour after treatment. If the mortality in control larvae was between 5 to 20 percent, Abbott's formula[13] was used correct the mortality with experimental larvae as given below:

$$\text{Corrected control mortality} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

Moribund larvae in the replicates were counted as dead. The software package 'ASSAY' (courtesy of Dr. C.F.Curtis, London School of Tropical Medicine and Hygiene, U.K) was used for dosage mortality regression analysis. Resistance ratio (RR) at lethal concentration levels (LC_{50}, LC_{90}, and LC_{95}) were calculated by previously described method [14].

To assess the efficacy of malathion for the management of *B. sphaericus* resistance in *Cx quinquefasciatus*, a technical grade malathion® (M/S Cyanamide India, Mumbai, 95% purity) (received from Defence Research and Development Establishment (DRDE), Gwalior, Madhya Pradesh, India) was used as toxin source. Malathion stock solution was prepared by titrating 0.5 ml of malathion containing 540 mg active ingredients (95% technical grade) in 499.5 ml of extrapure acetone (w/v). The aliquots of appropriate dilutions (two fold dilutions) ranging from 0.72 mg /lit to 0.011mg / lit were individually used for both *Bs* resistant and susceptible larval strains. These ranges of toxin were necessary to determine the susceptibility levels from 1.0% to 100% by keeping 7 concentrations. Bioassays were conducted in polythene cups. Larval food was provided to both larval strains and two replicates were placed for each bioassay. Experiments were repeated in three times at room temperature (~31 °C) and larval mortality was assessed 24 hour after treatment as recommended by WHO[15]. If the mortality in control larvae was between 5 to 20 percent, Abbott's formula[13] was used to correct the mortality as described above.

Results and Discussion

In the present study, the efficacy of malathion was evaluated against *B. sphaericus* resistant larvae of *Cx. quinquefasciatus*. Table 1 presents probit regression analysis for determining resistance ratio (RR) between resistant (GR) and susceptible (MS) strains by exposing the larval strains with *B. sphaericus* toxin. As shown in the table, the LC_{50}, LC_{90} and LC_{95} values in *Bs*- susceptible strain were 3.17, 21.84 and 37.76 mg *Bs* / lit respectively and it was found to be very low. Whereas, the LC_{50}, C_{90} and LC_{95} values in *Bs*-resistant strain were found to be very high in the levels of 3699, 22101.79 and 36691.26 mg *Bs* / lit respectively. The resistance ratio between GR and MS strains at three lethal concentration levels were 1166.9, 1011.99 and 971.7 folds respectively. Thus, the results indicated clearly that resistance was found to be very high in *Cx quinquefasciatus* larvae, when subjected to selection pressure with *Bs* toxin. It is worthwhile to mention here that resistant strain has been collected in the field (Gandhinagar, Kochi, Kerala) where, resistance at high level was reported [7]. The magnitude and rate of development of resistance were different in the calculated lethal

concentration levels (LC_{50} and LC_{90} and LC_{95}) and it is expected that there may be variations in RR, since, the percentage mortality of larvae between the test concentrations were high in GR strain than the MS strain (data not shown). However, statistically no significant difference in resistance ratio was observed in all lethal concentration levels (LC_{50} LC_{90} and LC_{95}) since, the fiducial limits were overlapping. It is presumed that selection pressure has increased general resistance by eliminating only the more susceptible individuals from the populations.

Table 1 also contains probit regression analysis data of the concentration-response relationship between GR and MS strains by treating the larvae with malathion. Resistance ratio at LC_{50}, LC_{90} and LC_{95} and their 95 % fiducial limits were mentioned. The LC_{50} LC_{90} and LC_{95} values for *Bs* - susceptible strain were 0.113, 0.55 and 0.87 mg *Bs* / lit respectively and did not differ significantly from LC_{50} LC_{90} and LC_{95} values for *Bs* resistant strain, which ranged from 0.108, 0.567 and 0.908 mg / lit respectively. The corresponding values of the resistance ratio were not significant and we detected only a slight degree of resistance (tolerance) at the lethal concentration levels. These changes in susceptibility may be caused by biological variations of the larvae used in bioassays or by uncontrollable experimental errors as reported earlier[16]. Therefore, we report the potency of an organophosphate group (malathion) to manage *Bs*- resistance in *Cx. quinquefasciatus* larvae.

Studies elsewhere have reported a high level of resistance to *B. sphaericus* toxin in *Culex* species[7,16]. Poopathi *et al.*[9] have also reported cross-resistance to different strains of *B. sphaericus* by *Cx. quinquefasciatus* larvae. In the present study, a critical consideration of resistance was evaluated on the toxic effect of *B. sphaericus* 2362 strain against *Cx. quinquefasciatus* . We found that resistance was very high in *Bs* resistant larvae. It was reported earlier that *B. sphaericus* resistance in *Culex pipiens pipiens* (French strain) was encoded by a single major recessive gene on linkage group I at 22.1 recombination units from the sex locus[16,18]. During mode of action, the crystal toxin from *B. sphaericus* fails to bind on the receptor site present on the midgut brush border membrane (MBBM) in resistant larvae, indicating that the receptors plays a vital roll for internalization of toxin[18]. This hypothesis is in agreement with the findings of other studies also[19,20]. Management of *Bs* resistance by malathion in mosquitoes in the present study is still need to be investigated to understand the roll of receptors, perhaps, these two toxins (*Bs* and malathion) mentioned in this study having independent modes of action. Hence, in the present study, we emphasis alternative application of insecticides for the management of *Bs* resistance in mosquito control operations.

Table 1– Response of *Culex quinquefasciatus* strains to malathion in larval bioassays

Toxins / Mosquito strains	Intercept	Slope ± SE	LC_{50} 48hr (mg/lit) (95% FL)	LC_{90} 48hr (mg/lit) (95% FL)	LC_{95} 48hr (mg/lit) (95% FL)	x^2 (df)	RR at LC_{50}[d] (folds)	RR at LC_{90}[d] (folds)	RR at LC_{95}[d] (folds)
Bacillus sphaericus 2362									
MS[b]	4.23	1.53±0 28	3 17 (3 85-2 61)[e]	21 84 (30 56-15 61)	37 76 (57 64-24 73)[e]	7 66 (4)			
GR[c]	0 89	1 65±0.25	3699 005 (4351 87-3144 1)	22101 79 (29584 29-16511 78)	36691 26 (52511 67-5637.15)	1 09 (4)	1166 9 (1667 4-816 6)	1011.9 (1895 2-540 3)	971 7 (2123 4-444.8)
Malathion (95% purity)									
MS	6.76	1.86±0 27	0 113 (0.131-0 097)	0 55 (0.72-0 42)	0 87 (1.20 - 0 63)	7 23 (4)			
GR	6 72	1.78±0 26	0 108 (0 125-0 093)	0.567 (0.747-0.430)	0 908 (1.27-0 65)	2 95 (4)	0 96 (1.29-0 71)	1 03 (1 78-0 60)	1.04 (2 02-0.54)

[a] *Bacillus sphaericus* 2362 (SPH-88) = 1500 International toxic units / mg *Bs* toxin

Strains of *Cx quinquefasciatus* [b] MS = Madurai susceptible strain , [c] GR = Gandhinagar resistant strain

[d] Resistance ratio = $\frac{LC_{50} / LC_{90} / LC_{95} \text{ from GR strains}}{LC_{50} / LC_{90} / LC_{95} \text{ from MS strains}}$

[e] 95% FL

Dose of exposure: *Bs* : 29.23 to 0 46 gm / lit and 38.97 to 0 61 mg / lit for *Bs* resistant and susceptible strains ; Malathion 0 72 mg / lit to 0 011 mg / lit for both *Bs* resistant and susceptible strains

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Amniogenesis in the Indian fruit bat, *Rousettus leschenaulti* (Megachiroptera-Pteropodidae)

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Abstract

Amniogenesis in *Rousettus leschenaulti* is by cavitation The primitive amniotic cavity is formed by apoptosis of cells in the center of the embryonic mass, coalescence of spaces developing in between the cells and radial rearrangement and polarization of the cells. Epiblast differentiates into a shield/floor and the amniotic ectoderm. The epiblastic roof becomes thin, single layered but persists. It gets an investment of mesoderm to form the definitive amnion.

(**Keywords :** foetal membrane/amniogenesis/fruit bat.)

Introduction

Embryogenesis in bats, as in other mammals involves the development of amnion yolk sac, allantois, and chorion (Perry[1]). A non-cellular, homogeneous eosinophilic and PAS-positive accessory membrane (Reichert's membrane) has been described in a few species of bats (Karim and Bhatnagar[2]). Mossman[3] emphasized the value of using foetal membrane characters as phylogenetic indicators among the major groups of eutherian mammals, and attributed their value to conservatism as compared to the development and morphological characters of the individual. Badwaik and Rasweiler[4] in a recent paper emphasized that the study of pregnancy-related processes and characteristics should help us in, efforts to sort out phylogenetic relationships within the Chiroptera and possibily with other mammalian orders. In the case of bats, however, the diversity in these processes and characteristics has often proved more confusing than helpful in this regard. This probably reflects the need for more information and analysis, rather than the existence of insurmountable obstacles to this line of enquiry. Comparative studies that attempt to explain how and why such diversity has developed within a closely related group, such as bats, can also help to understand similar processes or events in other mammals.

The present study is the first report on the amniogenesis in the Indian fruit bat, *Rousettus leschenaulti* (Desmarest). .

Materials and Methods

Sixty five female *Rousettus leschenaulti* carrying different developmental stages were collected during the reproductive cycle November-March and March-July from an underground tunnel near Bibika-Mukbara at Aurangabad and from a mine tunnel at Ramtek, Nagpur, India. The genitalia were fixed in different fixatives - alcoholic Bouin's, Rossman's fluid and neutral formalin. The gestation sacs were processed for paraffin embedding. 5-8 μm sections were stained with haematoxylin-eosin. Photomicrographs were taken with Leica M3 camera attached to the microscope.

Observations

Breeding Habits: *Rousettus leschenaulti* breeds twice in a year and exhibits postpartum estrus and pregnancy (Gopalakrishna and Choudhary[5]). The first cycle includes two waves: Copulation during early November and parturition in mid-March. Copulation in the third week of December and parturition by end of April or early May. The second cycle which is a postpartum cycle also includes two waves: Females copulate during the third week of March with delivery in the third week of July. It is not certain if all females carry pregnancy to term, there may be loss of embryos. Copulation in the last week of April or early May. Females invariably abort their embryos. The gestation period is of 125 days. The corpus luteum persists from one pregnancy to another and pregnancy alternates between the two successive cycles (Gopalakrishna[6]).

Amniogenesis: At the free bilaminar blastocyst stage in the uterus the embryonic mass shows intercellular spaces (Figs. 1 and 2). The bilaminar blastocyst expands and undergoes implantation in a preformed implantation chamber at the cranial end of the uterus (Fig. 3). Apoptosis (cells exhibit cytoplasmic shrinkage, nuclear condensation and fragmentation leading to cell death) of some of the cells in the . center of the embryonic mass (Figs. 3 and 4) occurs along with a radial rearrangement and polarization (Fig. 6) leading to the establishment of a large cavity - the primitive amniotic cavity. The cell debris filling the primitive amniotic cavity (Figs. 5, 8 and 9) disappears progressively. At first the primitive amniotic cavity is lined by a uniformly thick wall composed of three or four cell layers (Fig.6) on all the sides but soon the epiblast differentiates into a shield/floor and the amniotic ectoderm (Figs. 7, 8 and 9). With the expansion of the epiblast into a disc, the roof of the primitive amniotic cavity becomes thin, consisting of a single layer of flat cells (Fig. 8). The epiblastic roof persists and gets an investment of mesoderm to form the definitive amnion (Fig. 10). During later stages of development, the formation and the expansion of the exocoelom

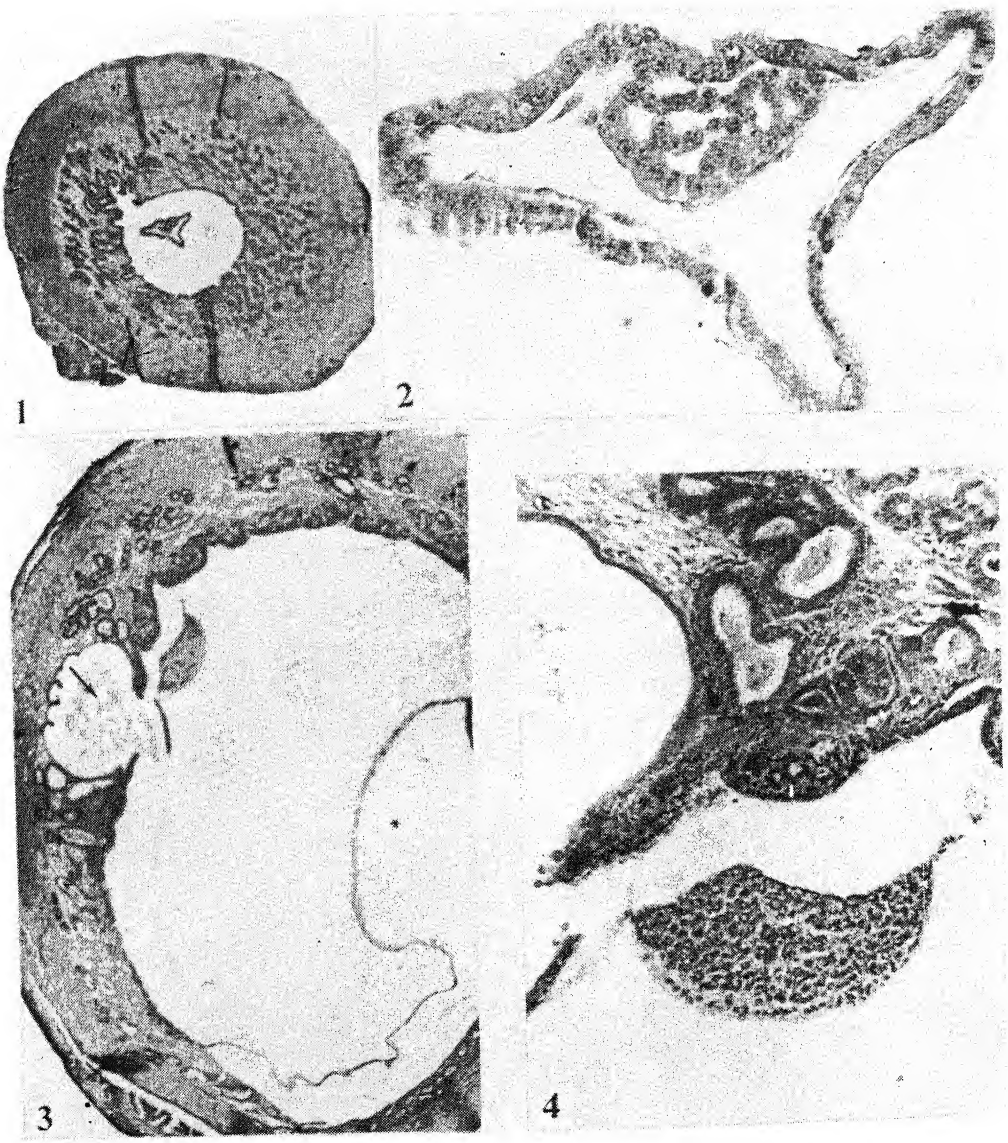

Fig. 1–T.S. cranial end of the uterus with a free bilaminar blastocyst (arrow) in the uterus. x 23

Fig. 2–Bilaminar blastocyst in figure 1 magnified to show the presence of the intercellular spaces (arrows) in the embryonic mass. en: endoderm; tr : trophoblast. x 240

Fig. 3–Superficially implanted bilaminar blastocyst with embryonic mass (arrowhead) oriented towards the opening of the Fallopian tube (arrow) into the uterus. The blastocyst wall is in contact on all the sides except a small abembryonic segment that hangs freely in the uterine lumen (*). y-s.c: yolk-sac cavity. x 36

Fig. 4–Part of figure 3 enlarged to show apoptosis of cells (arrows) in the embryonic mass. x 140

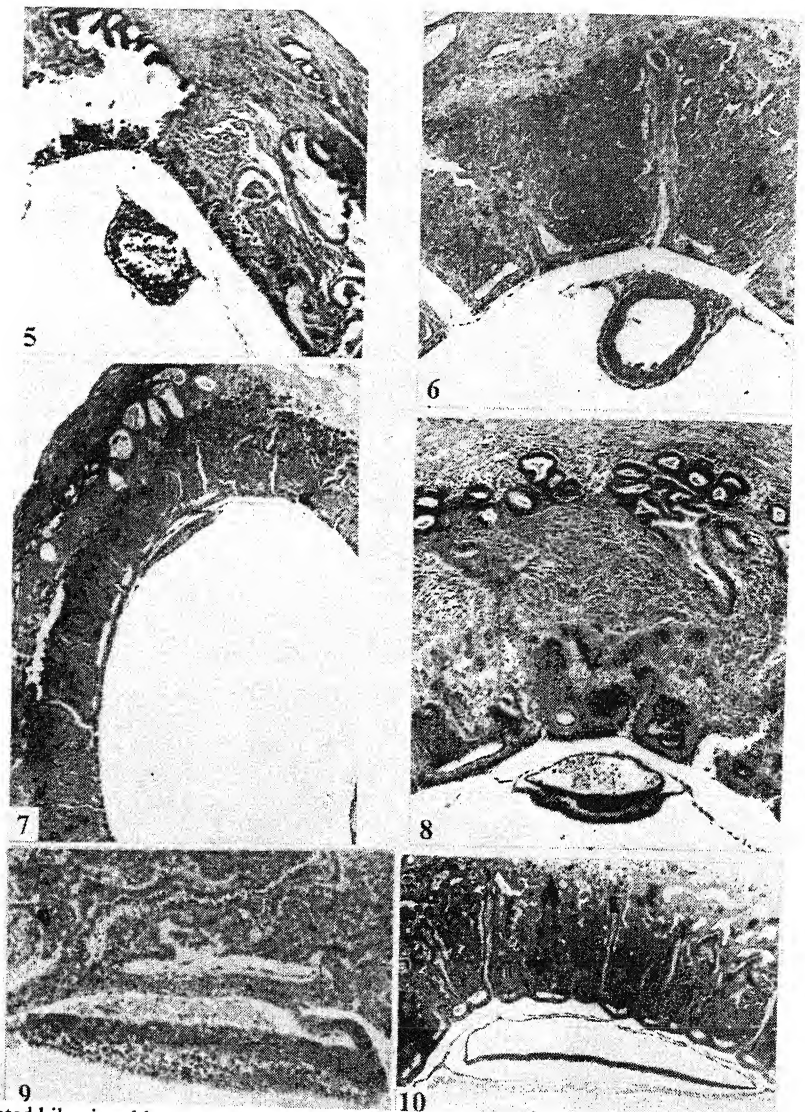

Fig. 5–Implanted bilaminar blastocyst with the embryonic mass showing degeneration of cells (arrow) and the formation of the primitive amniotic cavity (p.a.c). x 40

Fig. 6–Implanted bilaminar blastocyst with embryonic mass showing a large primitive amniotic cavity (p.a.c) formed by radial rearrangement of cells. At this stage the amniotic cavity is lined by uniformly thick wall comprising 3-4 cell layers (arrowhead). en: endoderm. x 50

Fig. 7–T.S. of the uterus containing a trilaminar blastocyst. The epiblast has differentiated into a shield and the amniotic ectoderm. m: mesoderm; y-s.c: yolk-sac cavity. x 24

Fig. 8–Embryonic area of a trilaminar blastocyst showing the epiblast differentiated into a shield/floor (arrow) and the roof consisting of a single layer of cells(arrowhead). The primitive amniotic cavity shows cellular debris (*). m: mesoderm. x 30

Fig. 9–Embryonic area of trilaminar blastocyst in figure 7 magnified (different section) to show the epiblast differentiated into a floor and a roof. The primitive amniotic cavity shows cellular debris (*).m: mesoderm. x 200

Fig. 10–Embryonic area of a trilaminar blastocyst showing lateral expansion of the epiblast. The definitive amnion is formed comprising of an inner epiblastic ectoderm (arrowhead) and an outer mesoderm (arrow). x 42

results in the separation of the amnion from its contact with the trophoblast, so that the amnion lies as a free bilaminar membrane. Thus amniogenesis in *Rousettus leschenaulti* occurs only by cavitation.

Discussion

The early embryonic development and development of the foetal membranes in *Rousettus leschenaulti* have been described by Karim[7]. The amnion is a thin, nonvascular membrane lining the amniotic cavity and the outer aspect is surrounded by mesoderm. In some bats (Karim and Bhatnagar[2]; Gopalakrishna and Karim[8]) the amnion becomes secondarily vascularized partly when it comes into contact with the vascularized wall of the allantois to form an amnio-allantoic membrane.

Four methods of amniogenesis have been described in bats (Karim and Bhatnagar[2]; Gopalakrishna and Karim[8]). These are:

i. Amniogenesis by cavitation :

This process involves both the apoptosis of cells within the embryonic mass and the coalescence of spaces that develop between the epiblast or by the radial rearrangement and polarization of epiblast in the inner cell mass giving rise to the primordial/primitive amniotic cavity. The epiblastic roof of the cavity so formed becomes thin with the expansion of the epiblast into a disc. The persistent epiblastic roof gets an investment of mesoderm to form the definitive amnion. In *Rousettus leschenaulti* amniogenesis is by cavitation - the primitive amniotic cavity is formed by development of intercellular spaces and coalescence of spaces in the embryonic mass, apoptosis of cells, radial rearrangement and polarization of the epiblast. The epiblast undergoes lateral expansion and differentiates into a shield/floor and the amniotic ectoderm. The definitive amnion is formed by the extension of mesoderm over the persistent epiblastic roof. Amniogenesis by cavitation is reported in *Thyroptera tricolor* (Wimsatt and Enders[9]), *Noctilio albiventris* (Rasweiler and Badwaik[10]), *Carollia perspicillata* (Badwaik, Rasweiler and Oliveira[11]), *Glossophaga soricina* (Hamlett[12]; Rasweiler[13]), *Scotophilus wroughtoni* (Gopalakrishna[14]), *Pteropus giganteus* (Moghe[15]), *Desmodus rotundus* (Wimsatt[16]).

ii. Primordial amniotic cavity formed by cavitation, the definitive amnion by folding :

The primordial/primitive amniotic cavity is formed as in i. above. The epiblastic roof of the primordial cavity is lost, creating a trophoepiblastic cavity. The definitive

amnion is subsequently formed by folds from the margins of the epiblast over which the mesoderm extends. This mode of amniogenesis is described for *Taphozous melanopogon* (Sandhu[17]), *Chaerephon plicata* (Gopalakrishna Pendharkar and Badwaik[18]) and *Molossus ater* (Rasweiler[19]).

iii. Amniogenesis by folding :

The inner cell mass transforms directly into an epiblastic plate. Between the epiblast and the overlying cytotrophoblast, the trophoepiblastic cavity is formed. Subsequent folds develop from the margins of the epiblastic plate which are reinforced by the extraembryonic mesoderm, thereby establishing the definitive amnion as in *Rhinopoma hardwickei* (Karim and Fazil[20]) and *Hipposideros lankadiva* (Khan[21]).

iv. Amniogenesis by coalescence :

The primordial/primitive amniotic cavity develops as a result of apoptosis of cells within the inner cell mass, which is pushed deeper into the blastocyst cavity by the proliferation of precociously-formed extraembryonic mesoderm. A mass of spongy tissue intervenes between the hollow embryonic mass and the cytotrophoblast layer. Folds develop from the margins of the roof of the primordial amniotic cavity forming a second cavity. For a short time, therefore, these two cavities stack over one another. Soon the two cavities become confluent with the breakdown of the roof. The roof of the secondary amniotic cavity constitutes the ectodermal component of the definitive amnion after it is enveloped by the extraembryonic mesoderm. This method of amnion formation if reported for *Cynopterus sphinx gangeticus* (Moghe[22]) and *Cynopterus marginatus* (Keibel[23]), *Haplonycteris fischeri* (Heideman[24]), *Pternochirus jagori* (Heideman and Powell[25]), *Otopteropus cartilogonodus* (Heideman, Cummings and Heaney[26]).

The comparative data on the embryology and foetal membranes in bats can be utilized to establish phylogenetic relationships and the affinities of the order Chiroptera with other eutherian mammals using cladistic methods (Gopalakrishna. and Karim[8]; Luckett[27]; Simmons[28]).

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Negative correlation between poly-ADP-ribosylation of mouse blood lymphocyte proteins and dimethylnitrosamine induced initiation of carcinogenesis as revealed by Slot- and Western blot immunoassays

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Abstract

Cancer detection, especially at an early stage, remains a serious challenge to researchers If detected in time the spread of cancer can be halted or reversed by appropriate interventions Existing predictive assays are not only cumbersome and expensive but also difficult to apply for mass screening consequently cancer related mortality is reported to be high. In this study efforts have been made to find a correlation between poly-ADP-ribosylation of blood lymphocyte proteins mice during initiation of carcinogenesis induced by a hepatocarcinogen, dimethylnitrosamine in a low dose chronic exposure condition Convenient, simple, inexpensive and sensitive Slot- and Western blot immunoassay has been employed to measure total cellular poly-ADP-ribosylation of lymphocyte proteins and that of individual lymphocyte proteins, respectively Results show significant lowering of total PAR of lymphocyte proteins within 2 weeks of DMN treatment as revealed by Slot blot immunoassay By Western blot analysis it has been bound that two lymphocyte proteins seemed to be the main causes for this observation. The preliminary report brings forward the possibility to use immunoassay of poly-ADP-ribosylation of blood lymphocytes as a possible predictive assay of initiation of carcinogenesis.

(**Keywords** . Ploy-ADP-ribosylation/Slot blot/Western blot/immunoprobing/Swiss albino mouse/carcinogenesis)

Introduction

Poly-ADP-ribosylation (PAR) of proteins, an enzyme catalyzed reversible metabolic process, is a post-translational modification. During this process ADP-

ribose moieties from endogenous nicotinamide adenine dinucleotide (NAD^+) are covalently bound to the target chromosomal proteins, particularly histones[1]. The reaction is enzyme catalyzed and totally reversible. Alterations in the PAR of chromosomal proteins change the existing interactions between the histones and DNA. This results in change in the chromatin super structure. Thus, the functional status of the chromatin is affected. Transformation of a normal cell to a cancerous cell involves a lot of molecular events such as gene amplification, changes in gene expression pattern, expression of neo-genes, shutdown of differentiation genes and dedifferentiation[2]. It is known that during the events listed above the structural organization of chromatin, comprising, the genetic material DNA and his tones and other chromosomal proteins, must change. One of the major metabolic factors proposed to felicitate this change is PAR of chromosomal proteins[3]. Therefore, it can be hypothesized that carcinogenesis induced structural changes in chromatin shall be reflected by the changes in PAR of chromosomal proteins. Earlier studies have shown that PAR of cellular proteins indeed show a negative correlation with DMN induced carcinogenesis in liver, target organ for dimethylnitrosamine (DMN)[4,5], spleen (an organ rich in reticuloendothelial cells and fenestrated capillaries)[6], and in asceitic cells[7]. A test based on biopsies either of the affected or any other organ needs surgical intervention making the test cumbersome. It also makes the test less practical to apply to any population being screened for cancer. Therefore, we have, in this report, attempted to ascertain whether or not blood lymphocytes could be used as an indicator of onset of carcinogenesis. The PAR of proteins of blood lymphocytes has been assayed during initiation of carcinogenesis induced by a hepatocarcinogen, DMN, in Swiss albino mice[8]. The aim of this study was to find out if a correlation existed between PAR of proteins of blood lymphocytes and carcinogenesis. A novel slot- and Western blot based immunoassay has been employed to assay PAR of the proteins[9,10].

Materials and Methods

Chemicals :

All chemicals were of analytical grade and were used without further purification. All required solutions were prepared in double distilled water.

Animals :

Swiss albino mice (Balb/C) were used in this investigation. The mice were maintained on standard mouse pellet and drinking water in a well-ventilated animal room.

Administration of DMN :

Young (6-8 weeks old) mice were exposed to DMN at a dose rate of 10 mg kg^{-1} body weight in drinking water in a chronic oral administration protocol over a period of 4 weeks. Mice were sacrificed for analysis by cervical dislocation at 0 (control), 1,2,3, and 4 weeks after initiation of the treatment.

Sample preparation :

Lymphocytes were isolated using Biocoll based centrifugation method from freshly drawn blood from the heart of the untreated (control) and DMN treated mice sacrificing them. Briefly, the lymphocytes fraction (10^6 cells) was washed and pelleted by centrifugation at 250_{xg} for 10 min. The pellets were suspended in 1 ml ice-cold lysis buffer. It was then vortex and left on ice for 30 min. It was spin at $5,000_{xg}$ for 10 min. The supernatant was used as the whole homogenate for slot- and Western blotting. Quantification of proteins was done by Brad ford's method.

Slot blotting :

Samples for slot blotting were heated in boiling water bath for 5 mins. to inactivate the cellular phosphatase enzyme in order to avoid its interference in a later step in the process. The samples were diluted with double-distilled H_2O to a final concentration of 3 μg protein is 100 μl. One hundred μl samples were loaded in the wells and slot blotted on nitrocellulose membrane (NCM; 0.45 μ) using Bio-dot sf apparatus connected to a vacuum pump.

Western blotting :

Samples for Western blotting were first subjected to 12% w/v SDS-polyacrylamide gel electrophoresis in a mini electrophoresis gel apparatus (25 V cm^{-1} constant, 60 min). The resolved proteins on the gel were electroblotted on NCM at 10°C in a BioRad transblot apparatus (100 V constant, 60 min) using Towbin buffer (25 mM tris-Cl buffer, pH 8.3, 192 mM glycine and 20% methanol).

Ink staining of blots :

The slot- and Western blotted NCM were stained with India ink (0.2%, 3-4 hrs) to visualize total proteins.

Immunoprobe assay of poly-ADP-ribosylation :

PAR of individual cellular proteins was detected by Western-blot immunoprobing[9,10] Briefly, the slot- or Western blots were incubated with in sequence, 5% non-fat dry milk at room temperature for 20-45 min, polyclonal anti-ADP-ribose antibody (1:1,500) overnight at 37°C and anti-rabbit IgG-alkaline phosphatase conjugate (1:7,500) at 37°C for 180 min. Each incubation step was punctuated by appropriate washing step(s). The bands on NC membrane were color developed with NBT/BCIP color developer[10].

Analysis of slot- and Western blots :

BioRad imaging densitometer and molecular analyst software 1D were used for capturing, quantification and analysis of the data. Mean intensity of bands (OD x mm^2) of 2-5 independent replicates (each replicate with 6 mice) was taken as the measure of PAR of cellular proteins (intensity $\propto$ PAR of proteins). Statistical analyses and plotting of graphs were done using Excel and Origin programs, respectively. P values ≤ 0.05 were taken as significant.

Results and Discussion

Human suffering from cancer continues unabated making it the single largest killer disease of humans. In spite of enormous efforts to combat cancer, the dreaded disease still remains an undaunting challenge to researchers world-wide. Due to extremely complex and largely unclear intricacies of cancer development process or carcinogenesis at molecular level, it has not been possible to develop one conclusive test for detection of cancer. Consequently, there are several pathological, morphological and biochemical tests which are currently being employed by clinicians to predict cancer in a patient. The tests in practice require sophisticated laboratories, thereby limiting its reach to common people, particularly in a large and poor country like India. A singular important factor in fight against cancer is its early detection. It has been clinically observed that cure of cancer in its early stages of growth could be as simple as treating most common diseases. Unfortunately, so far early detection of cancer has remained a problem Cancer grows stealthily within the body for usually a long period of time, extending to dozens of years, and makes its appearance felt only when it is too late. At a late stage other therapeutic modalities like radiotherapy, chemotherapy, etc. are the only avenues left. Clinical experiences show that the outcome of these therapeutic interventions at a late stage yields poor clinical efficacy. Therefore, to successfully fight the disease, early detection of cancer

is of outmost importance. Keeping this in mind, we have been making an attempt to develop a sensitive, reliable, simple and convenient test to predict cancer even at a very early stage.

It has been reported that the PAR reaction is intricately associated with carcinogenesis process induced by different carcinogens[3-6, 10,11]. Slot- and Western blot based immunoprobing using polyclonal antibody against physiological, heterogeneous ADP- ribose moieties has been reported to be a sensitive and convenient assay of cellular PAR[9 10]. A negative correlation was found between initiation of carcinogenesis and PAR of total cellular proteins or histone proteins[11]. Even ascites cells showed similar trend[7]. Therefore, it seems highly likely that PAR may predict the process of carcinogenesis. However, obtaining biopsies of affected organs for assaying PAR of proteins has obvious problems. With this in mind, blood lymphocytes were tested to find a correlation between PAR and carcinogenesis. The immunoblot assay developed in our laboratory has been used in this investigation since the assay is reported to be sensitive and reliable[10]. A known hepatocarcinogen, DMN, was administered on mice and their blood lymphocytes were tested for PAR. The examination was restricted to 4 weeks since, the process of initiation of carcinogenesis, an important and irreversible stage, falls in this period[6].

Fig. 1 shows the slot blot of samples obtained from control and DMN treated mice. Equal amounts of whole homogenate total proteins were slotted on the NCM as evident from even visual examination of the India ink stained NCM (Fig. 1-I). Its replica was immunoprobed showing different levels of ploly-ADP-ribosylated pro proteins (Fig. 1-II). Upon quantification, the almost straight but negative correlation between total PAR of lymphocyte proteins with progression of period of exposure to DMN was clear (Fig. 2). The DMN induced lowering of PAR of lymphocyte proteins was statistically significant from 2 weeks onwards reaching the level of about 50% of the control in 4 weeks. The samples were further analyzed by Western blot immunoprobing (Fig. 3). The lymphocyte protein profiles revealed by coomassie brilliant blue staining of the Western blot for control and DMN treated lymphocyte were expectedly similar (Fig. 3-1). Upon immunoprobing, however, it came out that only some proteins of the lymphocytes were poly-ADP-ribosylated. In general all DMN treated samples showed weaker PAR signal than the control (Fig, 3-II). Two protein bands showed a clear correlation between their PAR and progression of DMN treatment period (Fig. 3-II, arrow heads a & b). These two protein bands were quantified (Fig. 4). The lowering of PAR of these two lymphocyte proteins showed a clear negative correlation with period of DMN exposure (Fig. 4). The lowering was significant in 3rd and 4th weeks of treatment. In the 4th week of DMN treatment, both

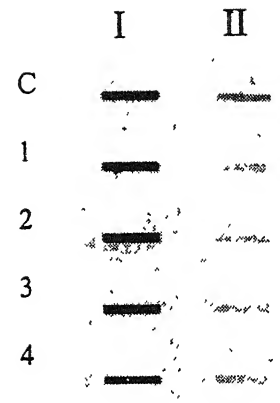

Fig 1–Slot blotted nitrocellulose membranes stained with India ink (I) and immunoprobed (II) showing blood lymphocyte whole homogenate samples of control, untreated mice (C) and 1 to 4 weeks DMN treated mice (see text for details)

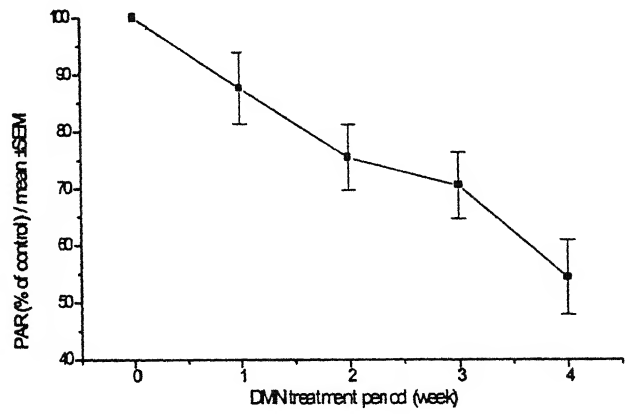

Fig. 2–Plot of total poly-ADP-ribosylated blood lymphocyte proteins from different samples as a function of DMN treatment period as quantified from immunoprobed slot blots (Fig. 1-II). Data at 2, 3 & 4 weeks were statistically significant ($p \leq 0\ 05$) as compared to the control

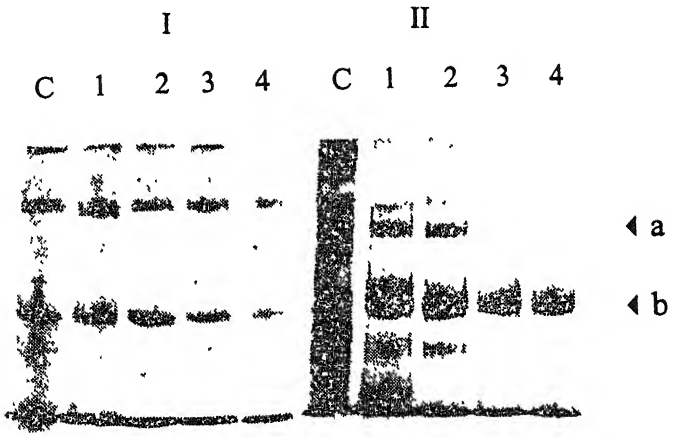

Fig. 3–Western blotted nitrocellulose membrane showing protein profile of lymphocyte whole homogenate samples stained with coomassie blue (I) and showing only poly-ADP-ribosylated proteins of lymphocytes after immunoprobing (II) in control, untreated mice (C) and 1 to 4 week DMN treated mice (see text for details). Arrowheads 'a' and 'b' are the protein bands, which have been analyzed further.

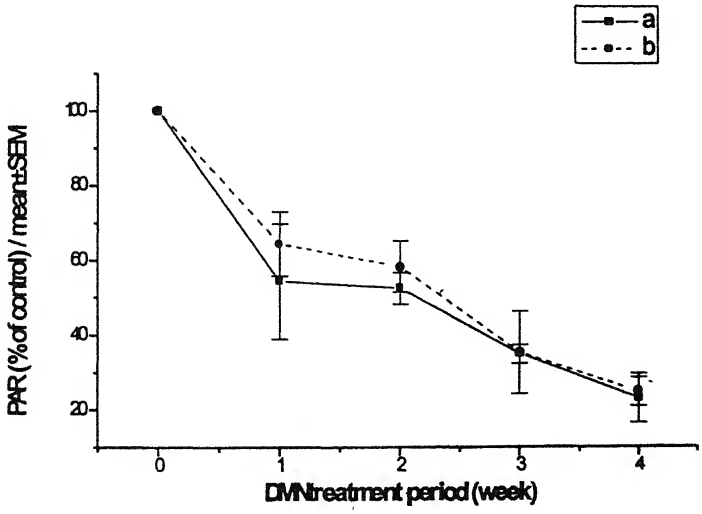

Fig. 4–Plot of poly-ADP-ribosylated blood lymphocyte protein 'a' and 'b' as a function of DMN treatment period as quantified from immunoprobed Western blots (Fig. 3-II) Data at 3 & 4 weeks were statistically significant ($p \leq 0.05$) as compared to the control

the proteins had about 25% PAR as compared to the controls While we have not identified the target proteins for PAR in lymphocytes, it is clear that there is progressive lowering of PAR of some proteins during initiation of DMN induced carcinogenesis Either total PAR of lymphocyte proteins (slot blot assay) or PAR of these two proteins (Western blot assay) showed negative correlation with period of DMN exposure. This indicates that both slot- and Western blot immunoprobe assays of PAR of blood lymphocyte proteins have great potential to become an indicator of initiation of carcinogenesis. More work is underway and attempts are planned to analyze human samples in the near future.

The etiological significance or a diagnostic value of poly-ADP-ribose antibodies is obvious albeit not exploited yet. Early detection of carcinogenesis remains a challenge world-wide and is especially relevant to countries like India where regular health check-up is poor. Using blood lymphocytes for predicting cancer in other organ, liver in the case of DMN induced carcinogenesis makes it further convenient. from these viewpoints, possible use of antigenic property of the ADP-ribose polymer as a diagnostic tool for early detection of carcinogenesis or mass screening of population is very attractive. The assay being non-radioactive, sensitive and quick adds to the possible advantages. Efforts are on to develop and standardize a convenient assay kit for measuring PAR of blood lymphocyte samples from human subjects using the polyclonal antibody, so that it may be used in mass screening of the population.

Acknowledgements

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Length-weight relationship of *Puntius sophore* (Ham.) from Allahabad region

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Abstract

The relationship between length and weight of *Puntius sophore* (Ham) was studied from Allahabad region The length and weight of this species follow the general parabolic equation

$$W=0\ 00006867\ L^{2\ 6683}$$

The value of regression coefficient 'n' (2 6683) was found less than cube of length and indicates that this species does not follow the cube law. A positive straight line correlation was found between these two variables with the value of correlation coefficient. r=0.95562. The analysis of variance proved the linearity of regression.

(**Keywords** · length-weight relationship/*Puntius sophore*/cube law)

Introduction

The length-weight relationship has various theoretical and practical application in fishery biology. Prediction of potential yield and determination of proper sizes of fish to harvest for maximum sustained yield are directly related to fish weight. These studies are mainly directed towards two objectives, first to establish a mathematical relationship between the two variables – length and weight, and second to know the variations from expected weight for various length groups. In certain cases this relationship is very useful in differentiating small taxonomic units, as variations may occur within populations of different localities[1,2]. The study of length-weight relationship is also known for its practical utility in fish management and conservation because these two variables are useful in deriving index of condition of fish studied. The length-weight relationship of several species of fishes has been studied by[3-11].

The present paper consists of the analysis of length-weight relationship in a sample of *Puntius sophore* from Allahabad region. The main purpose of the study was to find whether the fish grows isometrically according to cube law.

Materials and Methods

A total of 280 fishes ranging in length from 45 to 116 mm (total length) were used for the present study. However, their sexes were not considered separately. The

freshly caught specimens, after removing the moisture were measured for recording the length and weight. The lengths of fish were measured to the nearest millimeters from the tip of snout to the end of caudal fin and the weight was measured to the nearest grams.

The length-weight relationship was estimated by the method of least square by using parabolic equation as suggested by LeCren[1].

$$W=aL^n$$

or its logarithmic form, log W= Log a + n Log L

where,

W = weight of fish in grams,

L = length of fish in millimeters,

a = a constant

n = regression coefficient or an exponent expressing the relationship between W and L

Value of a and n were calculated empirically by the method of least squares. The parabolic relationship was expressed graphically by plotting the numerical values of length and weight and logarithmic relationship was expressed by plotting the calculated log weight against log length. The correlation or linearity of regression has been tested by analysis of variance.

Results and Discussion

The length-weight data were statistically analysed and the regression equation was computed as follows :

Parabolic from : $W = 0.00006867\ L^{2.6683}$

Logarithmic form : Log W = – 4.1632 + 2.66831 Log L (r = 0.9556)

The regression equation and the value of correlation coefficient, (r) (Table 1) is suggestive of a close relationship between length and weight of fish. The analysis of variance (Table 2) revealed that per unit increase in the total length was significant for

the per gram gain in the weight of fish. The perusal of length – weight equation derived for *P sophore* reflects the facts that the present species does not follow cube law. The weight of this species increases slightly less than the cube of its length as the value of regression coefficient 'n' was computed as 2.6683. The numerical values of weight when plotted against the length, gave a parabolic curve (Fig. 1), which showed an increase in length with the gradual increase in weight of the fish and straight line was observed with logarithmic values of lengths and weights (Fig. 2).

Table 1– Summary of regression analysis.

Regression Statistics	
Multiple R	0 955627
R Square	0 913222
Adjusted R Square	0 91291
Standard Error	0.062443
Observations	280

Table 2– Analysis of variance – length and weight of *P sophore*

Source	df	SS	MS	F
Regression	1	11.40717	11.40717	2925.584
Residual	278	1.083953	0 003899	
Total	279	12 49113		

The study of length-weight relationship in fishes is of primary importance in obtaining yield equations[12], in estimating number of fish landed and in comparing population in time and space[13]. The general expectation is that the weight of fish would vary as the cube of length[14-16] but actual relationship may depart significantly from this[1] as fishes change their form as they grow. However, the variations from isometric growth (n = 3) were found to be more[17] and for an ideal fish which maintains its shape throughout, without any change, the value of 'n' will be '3'[18]. Verghese[19], Talwar[20] and various other workers have shown that the value of regression coefficient 'n' either lies very close to the cube of length or differs significantly from this. The value of 'n' may change with locality, sex, maturity and with metamorphosis[21,1]. Under these circumstances the value other than three indicates allometric growth. According to Hile[22] and Martin[23], the values of 'n' usually fluctuate between 2.5 to 4.0 and in majority of cases the value was not equal to 3.

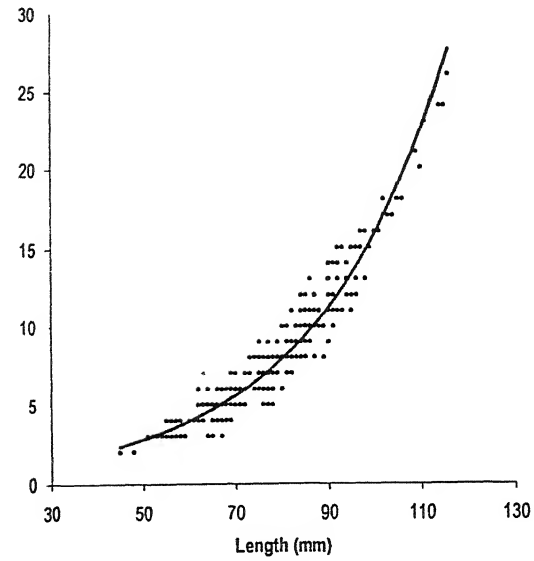

Fig 1– Length – weight relationship in *P sophore*

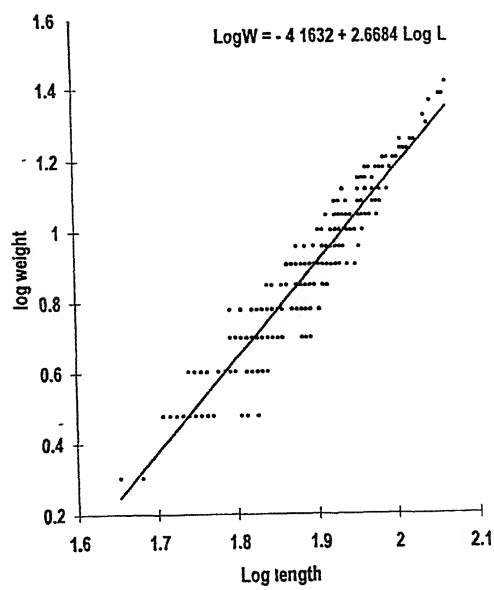

Fig. 2– Logarithmic length – weight relationship in *P sophore*

In the present study the value of n was found to be 2.6683. Thus there is departure from isometry (n = 3) in the length-weight relationship of *P. sophore.* The departure from cube law may be due to certain factors. According to Rounsefell and Everhart[24], as the specific gravity and shape or body outline of the fish is subjected to changes, the cube law does not necessarily hold good always. The seasonal changes notably the period during the immediately after spawning affects the length – weight relationship. Total weight of fish may also be altered by the weight of stomach content depending on the food ingested just before weighting[25]. This idea lends support to the value of n less than the cube law in the present analysis. Some investigators also found the value of n < 3 and do not agree with the cube law Rangaswamy[26] observed the value of n as 2.7234 for *Mugil cephalus,* Agarwal and Saxena[27] found the value of n as 2.18 in *Catla catla,* Soni and George[28] found the value of n = 2.759 for mudskippr *Boliopthalmus dentatus.* Rao and Rao[29] in Godavari river and Pathak[16] in the Loni reservoir have also observed the 'n' value less than 3 for major carp *Labeo calbasu.*

Since the regression coefficient (n =2.6683) of the length – weight relationship was found < 3, weight growth in *P. sophore* therefore, is positively allometric. The environmental conditions and water quality may be responsible for this departure. The total length was found closely or highly correlated to the total weight of the fish and increase in the length in highly significant for increase in weight.

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Status of benthic invertebrate community in river Godavari

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Abstract

Investigations on benthic community were conducted in river Godavari during pre-monsoon, monsoon and post-monsoon seasons. Results revealed maximum production of fauna during post-monsoon (7616 nos./m^2) and minimum in monsoon (3848 nos./m^2) Molluscs (gastropods and bivalves) dominated in middle (99.87%) and lower (98 45%) stretches while chironomids dominated in upper stretch (63 78%). Availability of food, water temperature, chemical status of soil, aquatic vegetation and nature of substratum have greatly influenced the availability of benthic community.

(**Keywords :** benthic fauna/mollusc/chironomid larvae/pulmonate)

Introduction

The need for detailed study of the bottom fauna, an important component of biotic communities, is self evident not only from the point of view of a better understanding of benthic dynamics but also from the fact that benthos constitute important food items of fishes. These animals are important not only in food chain leading to fishes but also in that they take part in biological water purification[1]. Studies on the availability and degree of utilization of such fauna in relation to food studies also help in demarcation of fishing grounds. River's benthic community has been studied very less when compared to lakes and reservoirs[2]. Despite the important place benthic organisms occupy in the food spectrum of the aquatic ecosystems, there is a dearth of published work on such studies except some observations made by Srivastava[3], Krishnamurth[4], Michael[5], Mandal and Moitra[6]. Gupta[7], Laal[8], Srivastava and Desai[9] and Kaushal and Prakash[10] in Indian waters.

The present investigation was conducted at river Godavari on the qualitative and quantitative composition of benthic community in relation to some environmental factors.

Materials and Methods

Area of study

River Godavari, (Fig. 1) the largest peninsular river originates from Deolali hills in Western Ghats near Nasik in Maharashtra at elevations ranging from 1219 to 1524 m (MSL). In its 1465 km long course, it traverses through the states of Maharashtra and Andhra Pradesh and opens into Bay of Bengal near Kakinada. The catchment of the river extends to an area of 312812 km^2, 48.6% of it falls in Maharashtra, 23.8% in Andhra Pradesh, 20.7% in Madhya Pradesh and the rest in Orissa and Karnataka. It includes densely forested high rain-fall zones of Western and Eastern Ghats and intensely cultivated dry regions of Deccan peninsula with low rain fall. The river is generally confined to within the banks and rarely overflows in its lower course. The flood plain lakes, characteristic of Ganga - Brahmaputra system, are absent here. The main tributaries are Pranahita, Indravati, Sabari and Manjira. Minor ones are Pravara, Puma and Maner.

Sampling Centres

Upper stretch (I) :

Five Centres were selected in this stretch viz., Someswar (Dist. Nasik). Kopargaon, Pravarasangam (Dist. Ahmednagar), Paithon (Dist. Aurangabad) and Nanded (Oist. N anded)

Middle stretch (II) :

Middle stretch starts from Andhra Pradesh border down the Eturunagaram. Sampling centres were: Khandakurti (Dt. Nizamabad), Khanapur (Dt. Adilabad), Dharmapuri, Manthani, Kaleswar (Dt. Karimnagar) and Eturunagaram (Dt. Warangal). Deep rocky or silty pools locally called 'Madugu' are found at frequent intervals along the river course in this stretch. One big pool of about 5 km length. known as 'Lanjan Madugu' is situated near Manthani.

Lower stretch (III):

This stretch extends from Parnasala (near Dummagudem) to the tidal regions of Yanam and Narsapur. The sampling centres were: Parnasala, Bhadrachalam. Kunavaram (Dt. Khammam), Polavaram (Dt. West Godavari), Rajahmundry, Kotipalli (Dt. east Godavari), Yanam (Pondicherry state) and Narsapur (Dt. west Godavari).

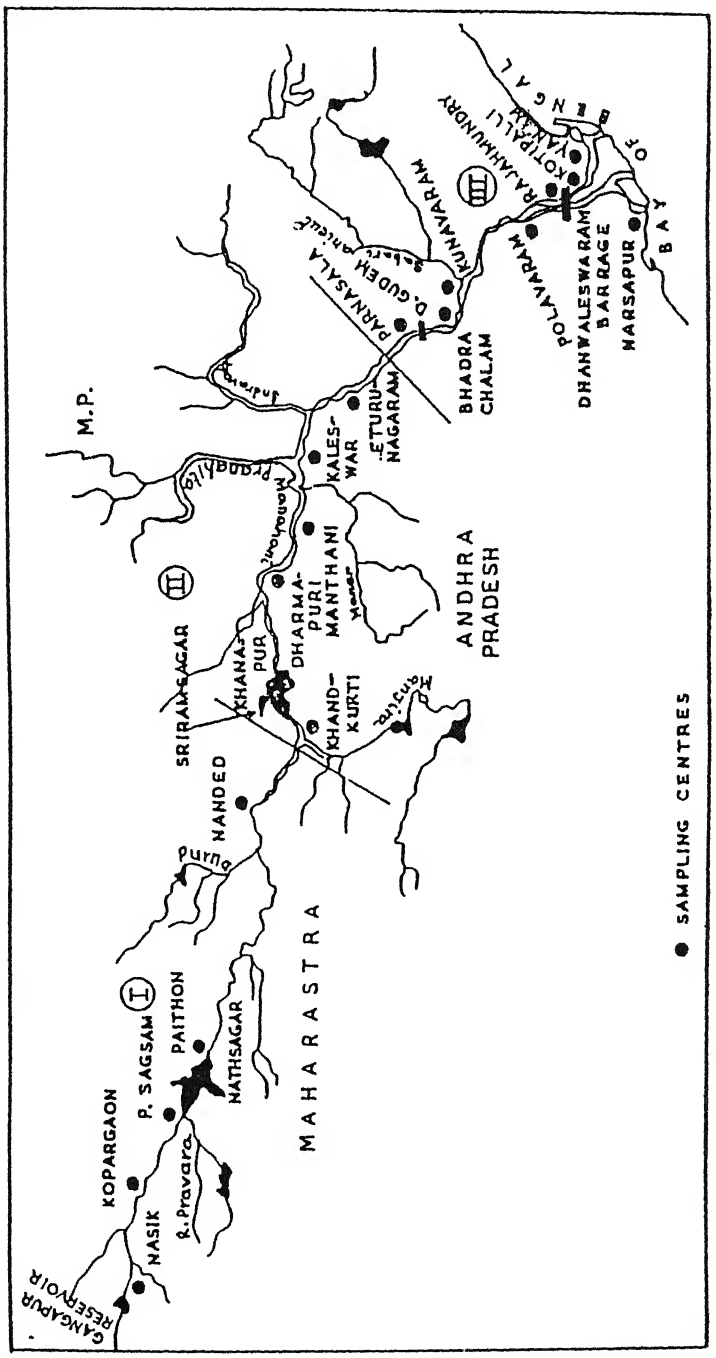

Fig 1–River Godawari,

The river course was divided into an upper, middle and lower stretches for detailed sampling. In upper stretch during pre-monsoon period sampling could not be undertaken due to very low water levels (almost dry) in the entire stretch of Maharashtra.

Benthic samples were collected during pre-monsoon, monsoon and post-monsoon seasons from different centres with a 15.2 x 15.2 cm (232 cm^2) Ekman's dredge. Samples were sieved with No4O sieve (250 mesh/cm^2) and were preserved in 5% formalin. Animals were sorted, counted and identified upto group level and wherever possible upto species level. For comparison of values the number of animals per haul were converted into values/m^2. Hydrological collections besides records of depth, temperature etc. were made as per APHA[11] and Jackson[12]. Sub-surface water samples were collected for this study.

Observations

Salient water and sediment characteristics

The water depth was 0.7 to 1.7 m in the upper stretch during monsoon and post- monsoon periods with mostly dry basin during pre-monsoon. In the middle stretch, barring deep pool like 'Lanjan Madugu' (9.5 m) water depth was generally in the range 1.8 to 3.0 m with little fluctuations (up to 3.0 m) in monsoon only in some sampling stations of the down middle stretch. Water depth was more in the anicut area like Rajahmundry as well as estuarine zone of Yanam and Narsapur in the over all range 10.4 to 12.5 m in monsoon and post-monsoon months with little lowering in summer months (Table 1). Water temperature varied from 23.0 - 31.5, 26.0 - 31.5 and 24.5 - 32.9° C in the upper, middle and lower stretches respectively with mean annual temperature fluctuated over a narrow range of 27.0 - 30.0 °c in the entire river course. Dissolved oxygen was uniformly high in upper and lower stretches (6.8-8.0 & 8.1-9.1 mg/l) and somewhat lower in the middle stretch (6.4-7.6 mg/l). A significant rise in pH during pre-monsoon followed by drop in monsoon and post-monsoon was noticed and pH was in the moderately alkaline range (7.4 - 8.2).

Table 1– Physico-chemical features of water of River Godavari

Centres	Depth (m)	Temp. (°C)	Transp. (cm)	DO (mgrl)	pH	Total alka. (mgrl)	Sp. condo (µS/cm)	TH (mg/l)	NO_3N (µg/l)	PO_4-P (µg/l)	SiO_2-Si (µg/l)
Nasik	1.7	27.2		7 7	7.6	103	415	98	54.5	115.5	17.8
	0.5-3.0	23-30.5	16-34	7.4-8 0	7.4- 7.8	92-114	400-430	76-120	32- 77	91-140	12.5-23.0
Kopargaon	0.7	28.2		8.0	8.0	100	485	118	36.5	180.0	17.5
	0.5-1.0	24-31.5	18-25	7.6- 7.4	7.7-8.4	84-116	380-591	96-140	35-38	130-230	11.8-23.2
P.sangam	1.5	29.0		7.0	7.4	99	472	116	31 5	74.0	13.8
	1.0-2.0	25-32.0	25.32	5.6-8.4	7.1-7.7	94-104	450-493	112-120	30-32	38-110	11.0-16.5
Paithan	1.9	28.2		6.8	7.6	106	493	102	26 0	95.0	14.2
	1.8-2.0	23.0-31.5	120-180	6.4- 7.2	7.3- 7.8	100-112	460-526	100-104	22-30	70-120	9.5-18.8
Nanded	1.5	28.3		7.1	7 5	119	525	116	35.0	99.5	13.1
	0.5-2.5	23.5-31.5	10-40	7.0- 7.2	7.2- 7.8	100-138	510-540	104-128	32-38	59-140	10.0-16.2
Kandkurti	3.0	28 7		6.4	7 7	137	463	120	24 0	80.0	12.4
	1.5-4.0	26.0-32.0	10-45	5.1- 7.7	7.5- 7.9	136-140	420-500	100-136	10-40	10-150	8.0-19.3
Khanapur	2.8	29.3		7.4	7.8	161	460	123	24.0	77.0	13.6
	1.0-3.5	28.0-31.5	15-180	7.2- 7.6	7.6-8.0	88-200	290-560	72-156	10-40	10-140	3.8-25.0
Dharmpuri	1.8	30.0		6.7	8.1	186	503	123	21.0	61.0	14 2
	0.7-2.5	29.0-31.5	17-75	6.2- 7.2	7.6-8.7	174-196	360-650	92-140	11-30	10- 140	5.8-19.3
L.madugu	9.5	29.4		6.6	8.2	191	605	145	32.0	60.0	11.1
	9.0-10.0	28.8-30.5	110-200	6.4-6.8	8.0-8.4	180-204	500-710	130-156	23-48	6-130	4.0-18.8

Table 1 Contd...

Table 1 Contd..

Kaleswar	2.5	30 0		6.8	8 2	158	490	127	23.0	83 0	11 7
	0.5-4.5	29 0-31 0	16-50	6.5- 7.0	7.9-8.6	130-180	390-590	98-144	10-38	34-140	4.5-19 3
E.nagaram	2.5	28 6		7.6	8.1	125	373	112	32.0	85.0	11.0
	1.3-3.5	28.0-29.5	6-150	6 4-8 8	7 8-8 4	100-148	3 10-460	80-140	25-42	40-130	5.0-19 3
Parnasala	6 6	27 0		9.1	8 2	116	277	105	33.0	92.0	8.2
	2.5-15.0	24.5-28.8	8-68	8.6-100	7.7-8.6	92-140	260-300	84-138	25-47	25-150	3 3-17.8
B.chalam	5.2	28.0		9.8	8 1	111	343	99	33.0	85 0	8.8
	1.8-8.0	25 0-30.0	6-120	8.0-11.5	7 7-8.4	88-136	260-500	72-136	25-44	30-150	3.8-17.5
Kunavaram	3.5	28.3		8.8	8.2	123	300	103	34.0	97.0	11 2
	1.8-5.6	27.0-30.0	6-120	8.4-9 5	7 7-8 6	92-140	260-340	76-120	22-45	50-140	2.4-16.3
Polavaram	4.5	30 0		8.7	7.9	96	270	84	30.0	100.0	10.4
	4.0-5.7	28.0-32.9	10-140	8.0-96	7.6-8.2	80-108	230-300	64-96	72-43	62-150	5 0-16.6
R.mundry	12.5	30.0		8 1	7.9	100	267	85	37.0	68.0	11.1
	11.0-15.0	28.0-32.0	8-120	7.4-8 8	7.6-8.2	87-120	245-295	71-104	)0-66	10-170	5 0-16.8
Kotipalli	11.3	30.0		8.7	7.8	103	347	95	31.0	65.0	7.9
	9.5-14.0	28.0-32.0	10-152	8.0-9.6	7.5-83	88-130	260-450	82-108	22-45	10-140	30-162
Yanam	10 4	28 0		8.9	7.8	104	21413	1724	31.0	62.0	1.8
	8.8-15.0	27.0-295	12-120	8.0-9.9	7.5-8.2	80-120	240-61200	72-4800	20-44	10-150	0.1-35
Narsapur	12.5	27.8		8.5	7.8	125	36643	2788	33.0	73.0	12.8
	10.2-17.0	175-29.0	10-90	6.9-10.2	75-80	100-142	330- 73600	98-5920	13-50	)5-150	38-/4.5

Sandy loam to sandy river bed was observed in the upper stretch with predominance of sand in the middle stretch (84-97%) except Lanjan madugu deep pool (loamy sand) and structure of river bed appears to have been modified due to weirs and barrages in the lower stretch barring Bhadrachalam, Konavaram and Polavaram. At Nasik, Paithan, Rajahmundry and Narsapur considerable amount (> 10%) of clay was recorded. Organic carbon (%) varied significantly amongst the centres at 0.15 - 0.52, 0.05 - 0.45 and 0.03 - 1.12 in the upper, middle and lower stretches respectively with maximum values noticed in the post-monsoon season (Table 2). Available nitrogen and available phosphorus (mg/l00 g) were very low in the range 1.10 - 27.3 and 0.23 - 1.02. Higher values of available nitrogen were found in postmonsoon ranging from 7.2 - 18.0,3.0 - 16.0 and 1.2 - 28.7 in the upper, middle and lower stretches. C/N ratio was in favourable range (6.4 -12.8) except at Narsapur where it was high (24.4).

Table 2– Sediment characteristics of river Godavari

*Locations	pH	Organic Carbon (%)	Total Nitrogen (%)	C/N Ratio	Available- N (mg/100g)	Available-P (mg/100g)
Nasik	7.8	0.44	0.06	8	13.5	0 45
	7 6 - 7 9	0 45-0.48	0.04 -0 09	5 3-11 3	12.0-15.2	0.40-0 50
Kopargaon	8 0	0 154	0 03	6 4	6.3	0 94
	7.8 - 8.1	0.10 - 0 20	0.02 - 0.03	5.0-7 5	5.5 - 7.2	0 80- 1,14
P. sangam	7.8	0 52	0.06	8 8	13	0.64
	7.7 - 7.9	0 50 - 0.54	0 05 - 0.07	7.7 - 10.0	10.2 -16.0	0.60 -0.68
Paithan	7 7	0 45	0.05	9.5	15	1.02
	7 5-7 8	0.40-0 50	0 04-0.05	9.0-10.0	12.0-18.0	0 90-1.20
Nanded	8	0 43	0.04	9.8	12.5	0.55
	7 9 - 8.1	0.40-0.46	0 03-0.05	9.2-10.8	10.5-14.0	0 50-0.58
Kandkurti	8	0 05	0 005	8.9	2.2	0 56
	7 9 - 8 2	0 04-0 06	0.004-0 007	8 0-10 0	1 5-3 0	0 50-0 62

Table 2 contd

Table 2 contd..

Khanapur	7.7	0 27	0 03	11	8 2	0 69
	7.5-7 8	0 24-0 30	0.02-0.03	10 0-12 0	7 0-9 0	0 50-0 80
Dharmapuri	7.7	0.28	0.03	11.5	8 5	0.3
	7 6-7 8	0 26-0 32	0 02-0 03	10.7-13.0	7.9 - 9 0	0 25-0 36
L. madugu	7.6	0 45	0 06	8 8	14.5	0 39
	7 5-7 6	0 38-0 56	0 04-0 07	8 0 - 9 5	12 9-16 0	0 36-0 42
E nagaram	7.9	0.21	0 03	8 6	7.1	0 3
	7.8 - 8 0	0 20-0 22	0 02-0 03	7.3-10 0	6 6 - 7 8	0.25-0 35
Parnasala	7 7	0.93	0 09	10	19	0 34
	7.6-7.8	0 65-1 25	0 06-0 13	9 6-10 8	17 0-22 0	0.25-0 46
B chalam	7 7	0 03	0 005	8	1 1	0 26
	7 5 - 7 8	0 03-0 04	0.003-0 006	6 7 - 10 0	0.9 - 1 2	0.20-0.30
Kunavaram	7.7	0.03	0 005	6 6	1 1	0 45
	7 5 - 7 8	0.20-0 04	0 003-0 006	5 0 - 8.0	0 9 - 1 2	0 10-0 58
Polavaram	7.8	0.76	0 07	11 3	13 6	0.25
	7.6 - 8 0	0 38-0.98	0.04-0 09	9 5-13 4	10 2-17 9	0 20-0.30
R mundry	7 9	1.08	0 11	9 8	27 3	0 39
	7 5 - 8 2	0 80-1.35	0 08-0.14	9 6-10.0	24 9-28 7	0 30-0 52
Kotipalli	7 9	0.58	0 05	12 8	13 4	0 23
	7.6 - 8.1	0.46-0 78	0.03-0 07	11 1-15.0	12.1-15 7	0 20-0 26
Yanam	8 1	0 4	0.05	8	14.2	0 81
	7 9-8 4	0.28-0.54	0.04-0.06	5.6-9.8	12.0-16 0	0 58-1.12
Narsapur	7 9	1.12	0.05	24 4	22 4	0 42
	7.7 - 8 2	0 82-1.36	0.04-0 06	20.5-30.0	20.7-24 2	0.30-0 62

Benthic communities

Data on distribution of the bottom animals are presented in Fig. 2 & 3. Molluscs (gastropods & bivalves) and chironomid larvae were generally recorded during pre-monsoon and monsoon seasons together with some nematodes and crustacean larvae. However, post-monsoon was devoid of chironomid and nematodes. Post-monsoon recorded maximum number of organisms (7616 nos./m^2 - mainly molluscs) followed by pre-monsoon (4692 nos./m^2) and monsoon (3848 nos./m^2).

Among mollusks, gastropods were in abundance as compared to bivalves. Gastropod percentage was 56.30 in pre-monsoon, 67.02 in monsoon and 64.70 in post-monsoon while bivalve remained at 38.44, 28.04 and 32.20 during pre-monsoon, monsoon and post-monsoon, respectively. Chironomids were observed occasionally at certain centres during pre-monsoon and monsoon seasons only together with some nematodes and crustacean larvae. Uniform distribution of benthic community was observed in the middle and lower stretch of the river throughout the seasons.

The invertebrate benthic fauna varied from 261 to 782 nos./m^2 in the upper stretch, 252 to 2631 nos./m^2 in the middle stretch and 26 to 2465 nos./m^2 in the lower stretch (Fig. 2 & 3). Centres with rich fauna are Dharmapuri (2631 nos./m^2) and Manthani (1122 nos./m^2) in the middle stretch, Polavaram (2465 nos/m^2) and Rajahmundry (1217 nos./m^2) in the lower riverine stretch and Yanam (1890 nos/m^2) in the estuarine zone. In the upper stretch Pravarasangam (739 nos./m^2) and Paithan (782 nos./m^2) showed higher density than other centres.

The bottom fauna was characterised by poor diversity with only molluscs occurring throughout the river course. Dipteran larvae were confined only to the upper stretch with predominant presence at Kopargaon and Pravarasangam. Molluscs (gastropods and bivalves) were dominant in middle and lower stretches accounting for 97 to 100% of the fauna. Gastropods formed the major segment of molluscan population and occurred in most of the centres. Common molluscs encountered were *Bellamya bengalensis, Thiara tuberculato. Brotia costula, Pila globosa, P virens, Lymnaea acuminata* (gastropods), *Corhiculo striatella, Lamellidens scutum and L. marginalis* (bivalves).

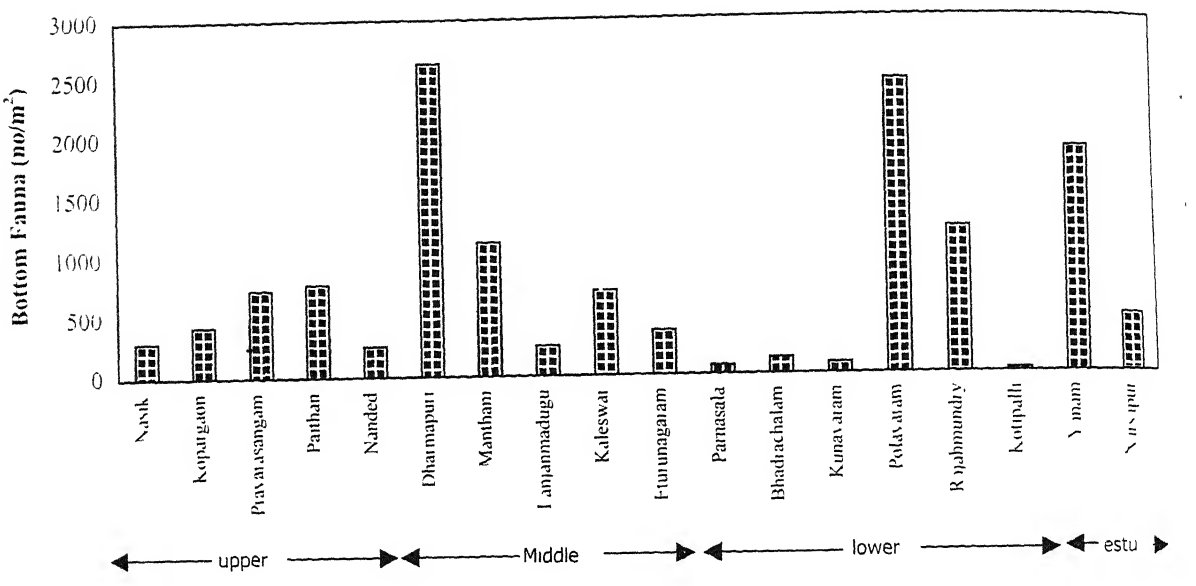

Fig 2–Spatial variation of benthic density in river Godawari

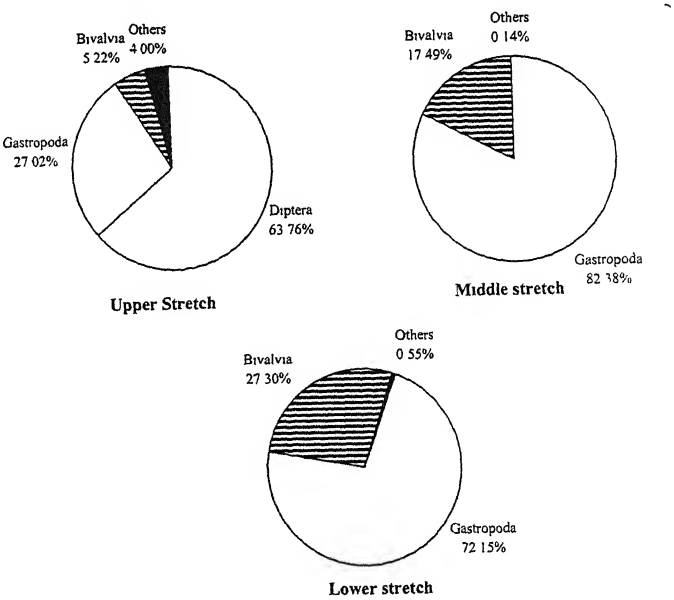

Fig. 3–Percentage composition of major bottom fauna groups in different stretches of river Godavari

Discussion

The distribution of benthic organisms varied between the seasons. Such variations of different forms from season to season and within the same season has been attributed to the disturbance of their habitat[2]. Such shifts in the fauna consequent in change of habitat affected by rain water incursion (erratic rain) were also evident in this study. Various factors are generally attributed to influence the qualitative and quantitative aspects of bottom fauna[13] Water temperature, oxygen, pH and chemical status of soil seems to influence the production of bottom macrofauna. It has been observed that bottom fauna[10] increases with the increase of nitrate, phosphate and organic status of soil. In the present study a marked increase of soil nutrients was noticed during post-monsoon after stabilization of allochthonous input which got entry into the riverine system during monsoon. Sediment organic carbon, nitrate and phosphates were at higher range during post-monsoon season and the same helped in increasing the bottom fauna.

It is well established that food plays an important role in the distribution of benthic organisms and nature of bottom has a selective influence on the quality of fauna[14]. Lundbeck[15] pointed out" a species appears when it has enough food, and disappears when oxygen condition makes its occurrence impossible". Gizinski[13] considered the dissolved oxygen content of water as one of the factors deciding the profoundal macrofauna in eutrophic lakes. Marlier[16] working on tropical rivers in Congo explained that the great abundance of benthic fauna were due to the availability of large quantities of food in suspension which was swept in by the current into the omnivorous creatures. Abundance of benthic community in post-monsoon may be due to the dominant planktonic population[17] (7616 u/l) during that period.

The stagnant conditions and low water levels probably favoured the establishment of dipteran larvae in the upper stretch. The peaks of these larval population are in coincidence with the rich phytoplankton population (99.03%)[17] in the river thereby providing nutrition to these larvae[2]. Maclachlan[18] and Soszka[19] emphasized the presence of chironomids in significant numbers in littoral zones of freshwater bodies. Some stretches of the river in Maharashtra were completely choked with aquatic weeds. This also may be the reason of chironomid presence in the upper stretch as studied by Rai and Datta Munshi[20] and Roy *et al*[21] that the chironomid larvae dominate in the weed infested sections of water bodies.

Ulfastrand[22] expressed that interplay of the factors is more important than that of the factors considered individually. This is thought to be the second important factor after the availability of food which primarily determines the dominance and seasonal

distribution of bottom fauna. In the present study, the predominance of molluscs (gastropods and bivalves) was observed throughout the period. However, excessive siltation as observed during monsoon seasons adversely affected the bivalvae population due to chocking their filter feeding systems. Similar observations have been made by Michael[5], Soszka[19] and Maclachlan[18]. Among the species of gastropods mostly belong to pulmonate group whose life on the weeds help in getting accessibility to the surface film for their respiration, oviposition and food[2]. In the lower stretch dense growth of weeds and grass occurred around islands between Rajahmnundry and Polavaram. Common plants were *Hydrilla verticillata, Potamogeton pectinatus, Vallisneria spiralis, Ceratophylum demersum, Typha elephantina Najas, Pistia* and *Spirodella.*

Nature of substratum was found to be an important factor in influencing the bottom fauna. It was seen that the centres like Dharmapuri (1850 nos./m^2) of mid stretch during pre-monsoon, Rajahmundry and Yanam of III stretch during monsoon (869, 1026 nos./m^2) and Eturunagaram (528 nos./m^2) and Polavaram (2244 nos./m^2) during post-monsoon with a substratum of sand and mud with weedy areas at regular intervals supported a dense molluscan population. Areas having sandy substratum supported a poor faunal element.

The inflowing rain water from catchment area would have settled the organic matter particularly in II and III stretch. During post-monsoon months, this settled organic matter would have decomposed favouring the growth of molluscs followed by their enhanced multiplication[10].

Low concentration of benthic organisms observed during monsoon season may be due to incoming flood water which might have mechanically dislodged them. Both number of species and density of benthic invertebrates were reduced during this period and only species capable of tolerating turbulance of water were found with regularity.

In the present study, maximum benthic fauna were noticed in November-December, a feature which corresponds to Rai[23]. From the foregoing observations it can be concluded that quality and quantity of benthic community is governed by various abiotic and biotic factors and proper understanding of interactions of different factors is of great importance as the same affect the production.

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Effect of sewage water on seed germination and seedling growth of *Vigna sinensis* and *Abelmoschus esculentus*

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Abstract

Sample taken from Chachar Nala, Allahabad (for sewage water) was analysed regarding temperature, pH, electrical conductance, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, carbon dioxide, organic carbon, total dissolved solids, total nitrogen, urea N, ammonia N, nitrate N, nitrite N, carbonate, bicarbonate chloride, phosphate, potassium, calcium, iron, copper, zinc and manganese *Vigna sinensis* (Lobia) and *Abelmoschus esculentus* (Bhindi) were selected for the study of germination and growth. The effect of sewage water on seed germination and seedling growth of vegetable crops were also analysed

(**Keywords** *Vigna sinensis*/*Abelmoschus esculentus*/soil)

Introduction

Sewage sludge is increasing day by day due to rapid increase in urbanization and industrialization. Sewage sludge contains many heavy metals (besides pathogens) which are toxic to animal and human being alike. A number of investigation on the impact of sewage on the physico-chemical and productive behaviour of soil such as saline and alkaline soil reclamation by sewage have been conducted.[1]

Utilization of sewage as fertilizer to increase crop production, improve the quality of soil and balance the economic condition etc. has been reported.

Mishra and Mishra[2] observed that the raw sewer water (sewage) and sludge contain beneficial (such as N, P and K) as well as toxic metals (such as Cd, Cr, Ni, Zn). A long term use of raw sewer water (sewage) for irrigating crops may cause metal accumulation in soils to such as extent that they may become toxic to plants.[3]

NEERI[4] reported the sewage BOD varies considerably and may be within a range of 200-500 mg/lit for irrigation purpose. According to standard proposed by FAO[5] maximum permissible limit for cadmium in irrigation water is 0.01 mg/lit. Azad et al.[6] also reported higher concentration of cadmium in effluent obtained from a group of

industries manufacturing metallic products. Thus, it may be concluded that these sewage water are not suitable for long term irrigation purpose from the view of cd content.

Effect of sewage on phytosociology and productive behaviour of some crop plants, e g. plant growth and dry matter production has been studied.[7] Others have reported the effect of sewage irrigation on the uptake of nitrogen, phosphorus and potassium in grain, straw and vegetative part of the plants.[8]

Sewage sludge has some manurial value mainly as a source of nitrogen, phosphorus and organic matter.[9] Heavy metal accumulate at soil surface through the sewage sludge application.

Materials and Methods

The Chachar Nala (for sewage water) discharges the entire water near Baluaghat in Yamuna about 5.5 km to the up stream of Sangam. The Nala contributes about 27% of the total pollution in Allahabad. Ten replicates, each of the two litre sample were collected at a time in glass stoppered bottles between 8 to 10 a.m. from the sampling stations.

Colour and odour of the sewage water were observed simply by naked eyes and nose by seeing or smelling it. The colour was light to dark black throughout the season except in rainy season. The odour was bad. The values of temperatures were 17.0°C and 31.5°C, minimum in winter season and maximum in summer season, respectively. Electronic conductivity meter was used for the measurement of electrical conductance. The average annual electrical conductance of this site was 883.4 μmhos/cm. The minimum and maximum E.C. recorded was 480.0 μmhos/cm in rainy season and 1230.0 μmhos/cm in summer season.

Organic carbon was determined by Walkey and Black's methods.[10]

An automatic oxygen analyser was used to analyse the DO and BOD content. The DO was 0.6 ppm in winter season (minimum) and 8.0 ppm in rainy season (maximum). The annual average DO was 3.0 ppm at this site. BOD values varied from 0.8 ppm (winter and summer season) to 8.6 ppm (rainy season) with an average value of 3.6 ppm. The Dichromate Reflux method was used to determine the chemical oxygen demand. COD varied from 3.1 ppm during rainy season (minimum) to 40.8 ppm during summer season (maximum). The average value was 18.9 ppm at this site. CO_2 fluctuated between 2.8 ppm during winter season (minimum) to 10.0 ppm during rainy season (maximum). The average value was 5.6 ppm at this site. Determination

of total solids was made by evaporation method and of suspended solids by using Gooch crusible.

Total nitrogen was analysed by micro-Kjeldahl method as described in A.P.H.A. Standard Methods. The minimum total nitrogen was 3.8 ppm in winter season. The maximum value recorded was 171.6 ppm in rainy season. The average value was 78.2 ppm at this site Phenol disulphonic acid method was applied for the analysis of nitrate, nitrite urea and ammonia-N (by direct nesslerization method) as described in A P H A standard methods.

The carbonate content was nil in winter season. The maximum content was observed during summer season. It was 6.3 ppm. The average value was 3.9 ppm. Chloride content was measured by 'Mohr' method. Stannous chloride method was adopted for the analysis of phosphate content. Na and K_2O were determined by flame photometer, Ca by oxalate method and Ca+Mg by titration with E.D.T.A (Ethylene diamine tetra acetate). Mg was obtained by the subtraction of Ca from Ca+Mg.[11] The minimum and maximum values of potassium recorded at this site were 13.6 ppm (rainy season) and 28.7 ppm (winter season), respectively. No zinc content was observed during rainy season The maximum content i.e. 0.4 ppm was in summer season with average value of 0.2 ppm.

The soil of Allahabad district is mostly alluvial. The soil of university farm is also alluvial The texture of the soil is clay loam. (Table 1).

Table 1– Physico-chemical properties of sewage water collected from Chachar Nala (Near Baluaghat), Allahabad

Sl No	Parameters	VALUES*			C.D.
		Minimum	Maximum	Average	
1	Colour		Black		
2	Odour		Bad		
3	Temp (°C)	17 0 W	31 5 S	25.6	6.8
4	pH	7 6 S	8.6 W	8 4	0.8
5	E C (μmhos/cm)	480 0 R	1230.0 S	883 4	5.8
6	Dissolved Oxygen	0.6 W	8 0 R	3 0	6.2
7	BOD	0.8 WS	8.6 R	3 6	1.6

Table 1 Contd..

Table 1 Contd.

8	COD	3.1 R	40 8 W	18 9	4 7
9	CO_2	2 8 W	10 0 R	5 6	7 2
10	Organic Corbon (%)	22 1 R	45 7 W	32 4	5 2
11	Total Nitrogen	3 8 W	171 6 R	78 2	5 2
12	Nitrate Nitrogen	0 0 W	54.0 R	13 4	1 9
13	Carbonate	0.0 W	6 3 S	3 9	2 1
14.	Bicarbonate	30 8 R	523 5 W	263 2	4 3
15	Chloride	31.2 R	135.2 S	86 2	7 2
16	Phosphate	1 3 W	4 4 S	1 9	1 8
17.	Potassium	13 6 R	28.7 W	22 6 W	2 4
18.	Iron	0.8 W	7.6 S	3 4	1 8
19	Copper	0 4 R	1 1 S	0 8	1 3
20	Zinc	0 0 R	0.4 S	0 2	1 2
21.	Manganese	0.0 R	0.6 S	0 3	1 2

*All the values (except pH) are given in ppm unless otherwise stated

**At 5% level of P

R=Rainy, W= Winter, S=Summer.

All pH measurement of soil samples were made with systonics digital pH meter using reference and glass electrode in 1:2.5 (soil : distilled water) suspension. Organic carbon in soil samples was determined by Walkley and Black's rapid titration method. Phosphate was determined by A.O.A.C. method. Total nitrogen of the soil samples was determined by Kjeldhal method. A neutral normal ammonium acetate was used for extracting the exchangeable cations and the soil left saturated with ammonium ions was digested with NaOH over a flame. The results were expressed in m.e./100 g of soil. Seven concentration of polluted water were made i.e. 0, 5, 10, 25, 50, 75 and 100 percent. Tap water (unclorinated) was used as control.

Seeds were soaked in the petridishes containing equal volume of different concentration of sewage water for 24 hours and then arranged for germination in other

petridishes on wet (with 15 ml of solution) filter paper at 27±2°C in dark and allowed to germinate for 1 week. At the end of one week, the percentage of seeds germinated, length of the primary root and shoot (by measuring it with scale in cm) were recorded. Change in fresh and dry (material was dried in oven at 80°C for 24 hours) weights of the seedling were also recorded. Second step of the study was completed in the pots. 10 seeds were sown in each pot. Same environmental condition was kept. Temperature was taken on average 26°C. At the end of 1st week, the percentage of seed germinated, was recorded. At the end of 2nd week, length of the root and shoot, fresh and dry weight were recorded. Average number of leaves were also counted. All the readings were taken after calculating the average (10 replicates). Similarly at the end of 3rd week all the readings were taken. The experiment was conducted in randomized block design with 7 treatments. The comparisons were made using the analysis of variance technique. The significance and nonsignificance of the treatments were tested with the help of F-test.

Results and Discussion

Due to pollution sewage water becomes black. Temperature of the water bodies increased by increasing the pollution level, i.e. sewage water (25.6°C) showed average temperature higher than the Ganga water. pH of the water increased by increasing the pollutants it was found that higher the pollution, greater was pH. Average COD value observed in sewage water was 18.9 ppm.

Sewage water showed lower carbonate content (3.9 ppm) and higher bicarbonate content (263.2 ppm). By increasing the pollution level in water bodies, carbonate was converted into bicarbonate and hence the value increased in more polluted water.

Sewage showed highest germination and growth on 25% concentration. From 50 to 100% concentration, germination and growth were less than control. The results of present study show that the temperature of sewage water varied with the variation in seasons. pH is the measure of the relative acidity or alkalinity and depicts the concentration of free hydrogen ions in the system. Electrolytes in a solution dissociate from the respective ions and impart conductivity to them. Lower pH value have been recorded for water bodies during rainy season.

Conductivity of water depends upon the concentration of ions and nutritional status. In civic sewage its value between 480-1230 μmhos/cm. In water bodies it varies with the season.[12] Low values of E.C. have been recorded during rainy season. The organic carbon content in water bodies are mainly due to dead organic materials.

Organic carbon was found highest in sewage. It varied with the seasons and ranged from 22 1-45 7% in civic sewage. Similar results have been reported by Singh and Bhowmick.[13]

Maintenance and distribution of biota in aquatic ecosystem largely depends upon the concentration of dissolved oxygen. High dissolved oxygen content is an indication of healthy system. The concentration of DO was found comparatively lower during winter season The low content of DO in raw sewage was due to high organic content. Similar findings were reported by Tripathi and Sikandar.[14] Biochemical oxygen demand is expressed in terms of amount of dissolved oxygen required in milligrams per litre for stabilizing the biodegradable organic matter by microorganisms of the sample under aerobic condition in a stated time. Similar findings were observed by Arora et al.[15] in sewage water.

Chemical oxygen demand (COD) is a measure of chemically oxidizable organic substance present in an aquatic system. Present observation regarding COD higher in civic sewage reveals the presence of higher quality of chemically oxidizable substances in the sewage. Similar results were recorded by Chattopadhya et al.[16] and Sikandar.[17] Seasons have been held to cause a pronounced effect on the amount of dissolved gases in water bodies.[18] Total dissolved solids (TDS) contains suspended particles, soil particles, discharged effluents, decomposed organic matter, dissolved solids, microscopic organisms etc. it controls the turbidity of water and varies with the season. The TDS during rainy season was recorded markedly higher than those of winter and summer seasons.

The amounts of nitrates and total nitrogen in water bodies have been shown to fluctuate with the season.[18,19] The N content of sewage water fluctuated between 3.8-171.6 ppm. This is a reflection of the massive amount of organic matter in the sewage. The sewage had greater amount of nitrate-N, it fluctuated between 0.0-54.0 ppm in sewage water. These findings indicate organic matter decomposition and nitrification during the flow and treatment. Similar results were observed by Chatterji et al.[20]

Amount of bicarbonate in sewage also fluctuated with season. Comparatively lower value were recorded during rainy season. The amount of bicarbonate varied in sewage effluent between 30.8-523.5 ppm. Higher values in sewage water show that pollutants tend to increase the amount of bicarbonates. pH of water body increases due to photosynthetic uptake of CO_2 available free or bound in HCO_3.

The amount of chloride in sewage fluctuated between 31.2-135.2 ppm. There is close relation between chloride and microbes of plankton in water bodies.[19]

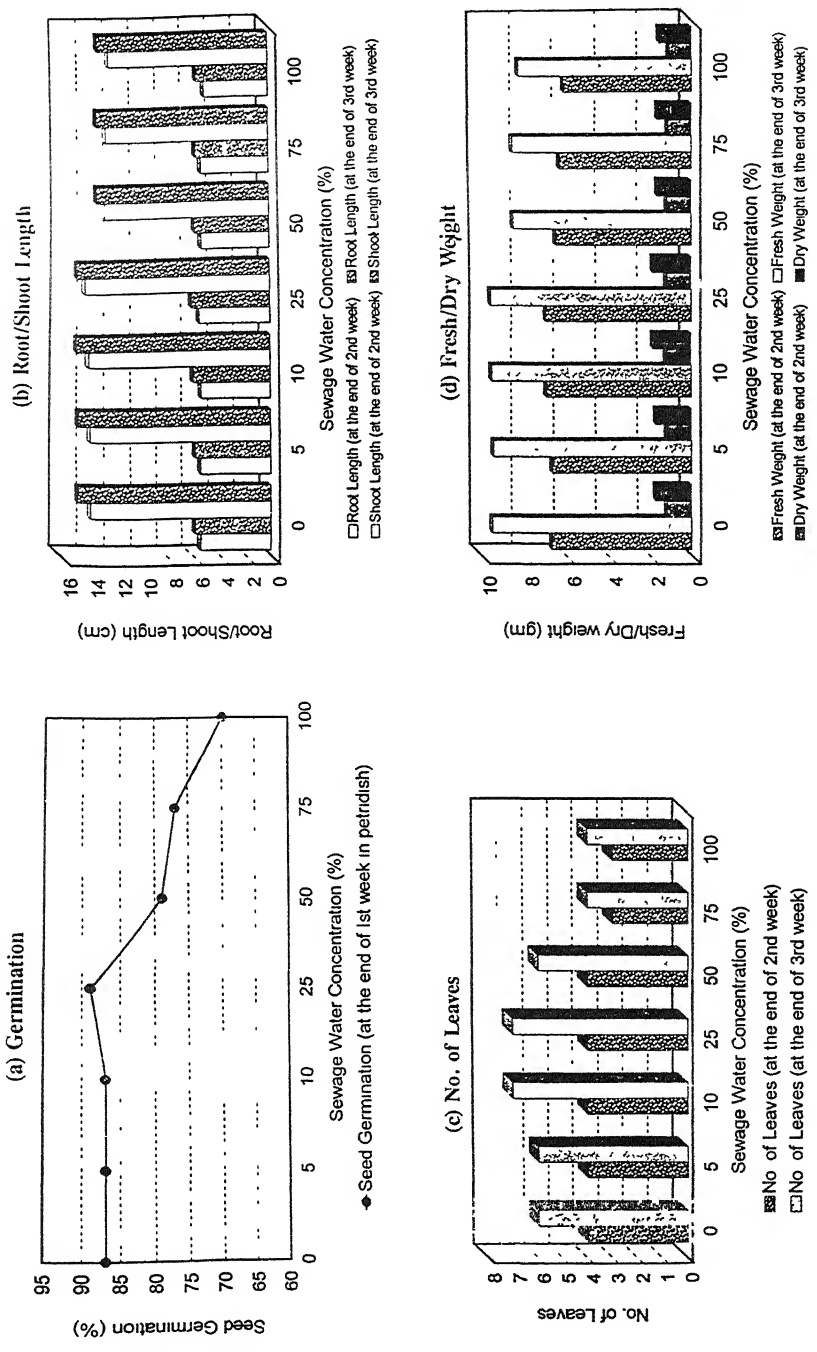

Fig 1–Effect of sewage water on seed germination and seeding growth of *Vigna sinensis*

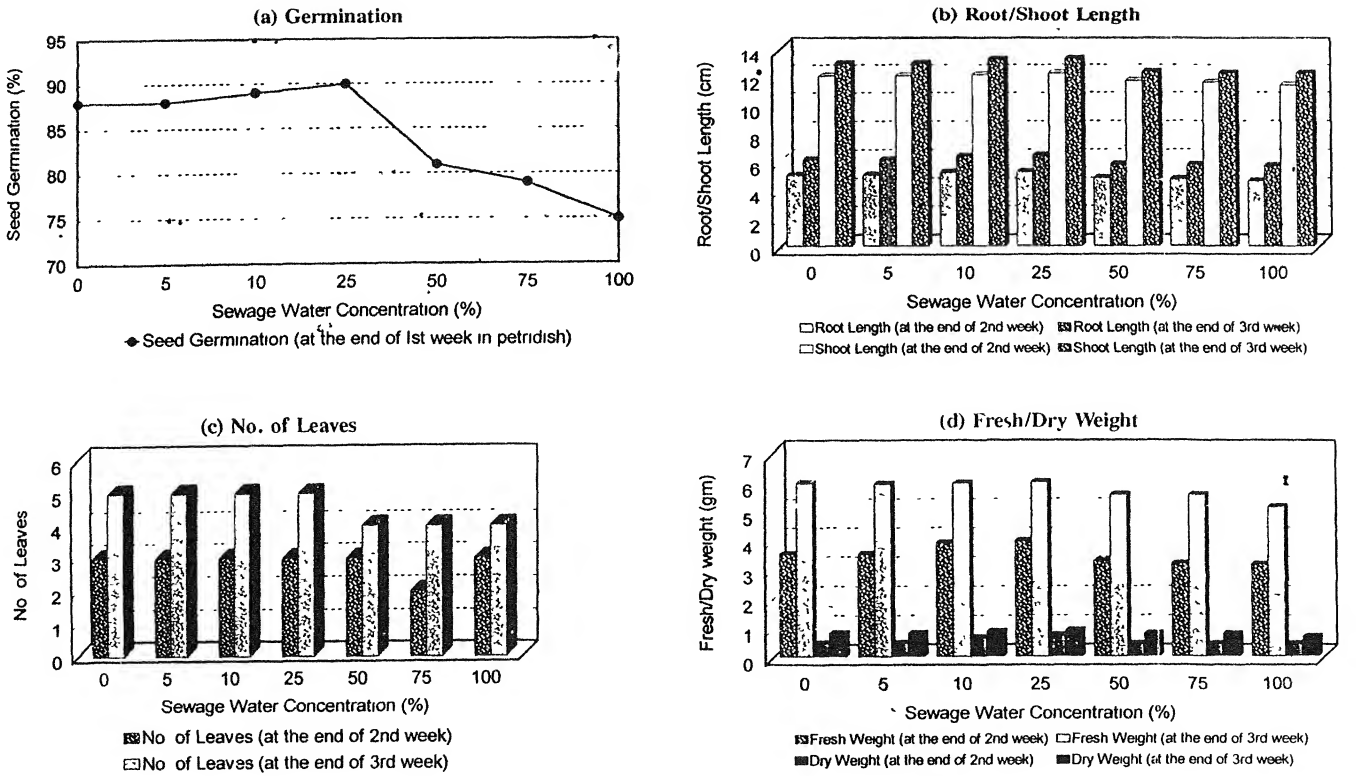

Fig 2–Effect of sewage water on seed germination and seeding growth of *Abelmoschus esculentus*

Phosphorus is one of most important nutrients which is required by the biota. Though in comparison to other major elements it is required in smaller amount, only the inorganic phosphorus as soluble orthophosphate plays an active role because over 85% of the total phosphorus usually exists in the bound organic form. In sewage water its concentration ranged between 1 5-1.8 ppm. The high values in sewage are supported by similar findings of Sikandar

In sewage water, there are a number of inhibitors. They may be heavy metals like Fe, Mn, Mg, Co, Hg etc. These may produce toxic substances which affect the germination of seeds The polluted water also contains, the toxic substances which affect directly seed germination. By these substances osmotic pressure increase and cause plasmolysis Due to this, cells may be destroyed. Lower (below 6) pH leads to dissolution of iron, aluminium and manganese in the water in large concentration enough to be toxic to the plant growth Sewage water is also concerned with the nutrients uptake, and it gave better response than control upto 25 percent in sewage water.

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Path coefficient analysis for seed yield in opium poppy *(Papaver somniferum* L.)

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Abstract

Correlation and Path coefficient analysis was determined in 22 selections of opium poppy *(P somniferum* L) for seed yield. Seed yield showed positive and significant correlation with plant height, leaves/plant, capsules/plant, stem diameter, capsule size, capsule weight/plant and husk yield/plant Capsule weight/plant had highest direct path (1 451) followed by plant height (0 378), capsule size (0 072) and stem diameter (0.033) towards seed yield. Opium yield and leaves/plant also contributed indirectly through capsule weight/plant, capsule size, capsules/plant and plant height

(**Keywords** : opium poppy/morphine/linoleic acid/seed yield/path analysis)

Introduction

The opium poppy is an important medicinal plant of diverse pharmacopoel uses. It is a rich source of morphine and seeds are used as byproduct in various preparations[1,2]. The opium poppy seeds are very nutritive. It contains about 50% oil with high Linoleic acid (70%), which reduces the blood cholesterol in human being[3,4,5]. Recently the demand of poppy seeds has gone up very high and thus development of high seed yielding varieties is necessitated. For enhancing the yield potential, component breeding for seed yield is important. Since correlation of component characters may vary both in magnitude and direction and tend to deviate the association of yield with its components, the path analysis, which splits correlation coefficient into direct and indirect effects and measures the relative importance of causal factors involved, is very effective in manipulating component traits. This study deals path coefficient analysis for seed yield in opium poppy.

Materials and Methods

In present investigation material comprised 22 genotypes developed through different breeding programs[4]. These 22 selections were evaluated in randomized block design with 4 replications during 2000-2001 under Initial Evaluation Trial. Each block

consisted of 22 plots with six rows per plot. The rows were 3 meter long. The spacing was 30 cm between rows and 10 cm within rows. Non-experimental rows were planted to check the border effects. Ten plants of each treatments per replication were selected at random before flowering to record observations on plant height (cm), leaves/plant, capsules/plant, stem diameter (cm), capsule size (cm^2), capsule weight/plant (g), seed yield/plant (g), husk yield/plant (g), opium yield/plant (mg) and morphine percentage.

Plot means per replication were used for statistical analysis. Path coefficient analysis was estimated according to Dewey and Lu[6].

Results and Discussion

Genotypic and phenotypic correlation among different component traits is presented in Table 1. The genotypic effects are higher than phenotypic effects for all the traits. At phenotypic level seed yield/plant showed significant positive association with husk yield/plant; plant height with leaves/plant, stem diameter and capsule weight/plant; capsules/plant with capsule weight/plant, seed yield/plant and husk yield/plant; stem diameter with capsule weight, seed yield and husk yield; capsule weight with seed yield and husk yield.

Seed yield/plant had positive and significant correlation with plant height, leaves/plant, capsules/plant, stem diameter, capsule size, capsule weight/plant and husk yield/plant at genotypic level. The capsule weight/plant revealed significant positive association with plant height, capsules/plant, stem diameter, capsule size, seed yield/plant and husk yield/plant, which clearly indicates that higher capsule weight/plant increases seed yield and husk yield/plant. The capsules/plant, capsule size, capsule weight/plant and husk yield/plant were positively and significantly associated among themselves and their positive association with seed yield indicate that the selection of component traits jointly or individually may enhance the production of seed yield/plant. Plant height showed significant positive correlation with leaves/plant, stem diameter, capsule weight/plant and husk yield/plant. However, the estimates of correlation alone, is often misleading due to mutual cancellation of component traits, hence, much reliance can not be placed. So the genotypic correlation partitioned into direct and indirect effects towards their relative contribution of the component traits through path analysis. Path coefficient analysis of seed yield with component traits was estimated and presented in Table-2. Capsule weight, which had highest genotypic correlation with seed yield (0.972) exhibited highest direct path (1.451) and also indirectly contributed to seed yield via leaves/plant, capsule size, stem diameter and morphine content. Plant height having positive significant correlation

Table 1– Genotypic and Phenotypic (parenthesis) correlation coefficients among different traits in opium poppy *(P somniferum* L)

Characters	Plant Height (cm)	Leaves/ plant	Capsules/ plant	Stem Dia-meter (cm)	Capsule size (cm^2)	Capsule weight/plant(g)	Husk yield/ Plant (g)	Opium yield/ plant (g)	Morphine (%)
Seed yield/plant	0 51 *	0 42*	0.59**	0 71 **	0 44*	0 97*	0 88**	0 22	-0.05
	(0.40)	(0.27)	(0.46*)	(0.53*)	(0 35)	(0.86**)	(0 44*)	(0 19)	-0.07
Plant height		0 67**	-0.21	0 59**	0 32	0 53*	0 47*	-0 12	0.26
		(0 53*)	(-0 19)	(0 52*)	(0.30)	(0.45*)	(0 32)	(-0 11)	(0.26)
Leave/plant			-0 14	0.37	-0 28)	0.32	0 12	-0 03	0 19
			(-0.12)	(0.31)	(-0 19)	(0 26)	(0 14)	(-0 01)	(0.22)
Capsules/plant				0 21	0 17	0.66**	0 73**	0 29	-0 17
				(0 14)	(0.11)	(0.58**)	(0.56**)	(0 24)	(-0 09)
Stem diameter					0 37	0 68**	0 56**	0 14	0.01
					(0.26)	(0 58**)	(0 42*)	(0 10)	(0 04)
Capsule size						0 50*	0.54**	0.02	0.28
						(0.40)	(0 32)	(0 04)	(0.18)
Capsule weight/plant							0 94**	0 23	-0.04
							(0.82**)	(0.20)	(-0.02)
Husk yield/plant								0.21	-0.03
								(0.15)	(0 06)
Opium yield/plant									-0.507*
									(-0.41 *)

**, *Significant at I % and 5% respectively

Table 2– Path Coefficient analysis for seed yield in opium poppy *(Papaver somniferum* L.)

Characters	Plant height (cm)	Leaves/ plant	Capsules /plant	Stem Diameter (cm)	Capsule size (cm^2)	Capsule weight (g)	Husk yield/ Plant (g)	Opium yield/ plant (g)	Morphine (%)	rg(seed yield/plant)
Plant Height	**0.378**	-0.150	0.052	0 031	0.023	0 309	-0.121	-0 005	-0.006	0 511 *
Leaves/plant	0 255	**-0.222**	0.034	0.012	-0 021	0 400	-0 032	0.001	-0 004	0 425*
Capsules/plant	0 081	-0.031	**-0.242**	0.007	0 012	0 958	-0 187	-0 012	0 004	0.590**
Stem Diameter	-0.225	0.083	-0.051	**0.033**	0 026	0 993	-0.144	-0.006	0 000	0 709**
Capsule size	-0 121	-0 064	-0.042	0 012	**0.072**	0 731	-0 139	-0 001	-0 006	0 443*
Capsule weight	-0 199	0 071	-0 160	0 023	0.036	**1.451**	-0 241	-0 009	0 001	0 972**
Husk yield/plant	-0 178	0 028	-0 177	0 019	0.039	1 369	**-0.256**	-0 009	0 001	0 885**
Opium yield/plant	0 047	-0 006	-0 070	0 005	0.001	0 329	-0 054	**-0.042**	0 011	0.220
Morphine	-0 122	0 043	0 041	0.000	0 020	-0 064	0 008	0 0021	**0.021**	-0 051

Residual effect = -0.0022

rg = Genotypic correlation.

(0.511) with seed yield contributed next highest direct path towards seed yield (0.378). It also had positive indirect path via capsule weight/plant, capsules/plant, stem diameter and capsule size. Leaves/plant had negative direct effect but indirectly contributed towards seed yield via capsule weight/plant (0.400), plant height (0.255), capsules/plant (0.034) and stem diameter (0.012). Significant positive correlation between leaves/plant and seed yield (0.425) also confirmed the present findings and nullified the negative direct path towards seed yield. The indirect contribution of leaves/plant via capsules/plant, capsule weight/plant and plant height and its positive significant correlation with seed yield indicated that since the leaves are responsible for photosynthetic activity, the increase in leaves/plant may increase seed production via these component traits. This is in consonance with earlier suggestions of Singh and Khanna[7]. Similarly the capsules/plant and husk yield/plant exhibited negative direct path towards yield but both indirectly affected seed yield via capsule weight and capsule size.

Capsule size, though had significant genotypic correlation but exhibited very low positive direct path towards seed yield. However, the low direct path effect was counter balanced via high indirect effect of capsule weight (0.731) and stem diameter (0.012) with positive significant genotypic correlation. The negative direct path of opium yield and low positive genotypic correlation with seed yield indicated that there was no direct bearing of opium yield to seed yield. Similar findings were also reported for different workers[9,10,11,12]. However, it contributed indirectly to seed yield via capsule weight/plant, capsule size, stem diameter and plant height. This indicates that though selection for opium yield is not directly exercised in the field (primary selection) but in laboratory (secondary selection) it contributed much towards seed yield via above component traits and thus high opium producing genotypes should be considered for increased seed yield[7,8].

The present study conclude that the capsule weight/plant, capsule size, plant height, stem diameter, leaves/plant and opium yield were important contributors towards seed yield on the basis of path analysis and genotypic and phenotypic correlation and hence due emphasis should be given to these traits in selection programme for increased seed yield. For component breeding, selection based on multiple characters might be appreciably used in the present set of material.

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Effect of spirulina on the larval and cocoon characters of the silkworm, *Bombyx mori* L.

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Abstract

Five different concentrations of spirulina *viz*, 10, 50,100, 150 and 200 ppm were supplemented through mulberry leaf as feed to 5th instar bivoltine pure (NB_4D_2) silkworm, *Bombyx mori* Linn, and the effects on larval duration, pupation rate, cocoon characters and silk-index were studied Among the concentrations studied 100-150 ppm concentrations significantly reduced the larval duration and enhanced the pupation rate, single cocoon and shell weights, shell ratio (19 90-20 48%) and silk-index (21-23%) compared to that of control.

(Keywords : spirulina/larva/cocoon/silkworm)

Nutrition is the most important growth regulating factor in silkworm, *Bombyx mori* L. Being a monophagous insect, it derives almost all the nutrients essential for its growth, rate of development and economic characters from mulberry leaf alone. Silk proteins in silk insect are directly derived from broad spectrum of proteins and amino acids available in mulberry leaves[1]. Amino acids are considered to be essential for silkworm growth[2]. Improvement in quality and quantity of silk by enriched mulberry leaves with organic, inorganic and antibiotics supplements has been tried by many workers[3-7]. These studies revealed that feeding of proteins, amino acids, vitamins and antibiotics through mulberry leaves have improved larval weight, growth, fecundity and silk contents. Soyabeans rich in protein (38-42%) improved the larval and cocoon characters[8]. However, no information is available on the possible role of spirulina, which is widely available in Indian market in different brands such as Recolina, Natoxid, Sunora spirulina and Multinal, on the larval and cocoon characters of silkworm, *Bombyx mori* L. Specific dose requirement of the different nutrients and vitamins suggests their specificity for various metabolic functions[9]. Hence, in the present study five different doses of spirulina were supplemented to mulberry leaf as a feed to silkworm to find out its effect on larval and cocoon characters of silkworm, *B. mori* L.

Spirulina is a blue-green algae and is a source of single cell protein (SCP). Its protein yield per unit area is the highest among protein yielding crops. It is rich in proteins (62.5- 71.0%), lipids (6.8%), carbohydrates (15-17%), crude fibre (0.02-

0.09%) and ash (7-9%)[10]. In addition, it contained 18 aminoacids and some other vital vitamins like, Biotin, Tocopherol, Thiamine, Riboflavin, Niacin, Folic acid, Pyridoxic acid, β-carotene and vitamin B_{12} etc[11].

For this investigation a bivoltine race of *B. mori* L., namely NB_4D_2 was obtained from the Central Silk Board, National Silkworm Seed Production Centre, Bangalore (India). Six grams of newly hatched silkworm larvae were reared under standard laboratory conditions adopting a general rearing method[12]. The most important effect of any physical and chemical agents on *B. mori* is in the fifth instar, when the larvae is distributed in metamorphosis and is not able to spin the cocoon[13]. Hence, fifth instar larvae were grouped into six treatments of three replications, each containing 500 worms. A known quantity of spirulina (recolina brand) was dissolved in distilled water and diluted to 10, 50, 100, 150 and 200 ppm in distilled water. Freshly collected mulberry leaves from the K-2 variety mulberry garden were soaked in the above concentrations for ten minutes individually and dried at room temperature (26 ± 1°C). The control group was fed with mulberry leaves soaked in distilled water. Feeding was given three times a day at eight hour intervals.

Mature translucent larvae were mounted on bomboo made mountages locally called chandrikaes (1.8 x 1.2 m). Cocoons were harvested after 5-6 days of spinning. 100 cocoons of each treatment were collected and weighed on an electric balance randomly. The shell weight was determined for cocoons selected for cocoon weight. The shell ration and silk-index were determined[14] Six important parameters *viz.*, larval duration, pupation rate, single cocoon weight, single shell weight, shell ratio and silk index were analysed in treated and control groups. The results obtained were subjected to statistical analysis[15] and are presented in Table 1.

$$\textit{Shell-ration (SR\%)} = \frac{\textit{weight of cocoon shell}}{\textit{weight of whole cocoon}} \; X\,100$$

$$\textit{Silk-index} = \frac{\textit{Average cocoon shell weight of treatment}}{\textit{Average cocoon shell of control}} \; X\,100$$

Table 1– Effect of spirulina on larval and cocoon characters of bivoltine silkworm, *B mori* Linn NB_4D_2 race

DOSAGE (ppm)	V Instar duration Day hrs	Pupation rate (%)	Single		Shell ratio (%)	Silk Index
			Cocoon weight (g)	Shell weight (g)		
10	9.17 (0 0)	72 70** (3.06)	1.761 (0 23)	0 335 (2.45)	19.03** (2.09)	1 03 (3 0)
50	9 12 (-0 55)	74.30** (5 33)	1 786** (1 65)	0 346** (3.86)	19 36 (3 86)	1.06 (6 0)
100	8 16* (-11 02)	78 53** (11 33)	1 924** (9 51)	0 394** (20.49)	20.48** (9 87)	1 21 (21)
150	8 06* (-12 10)	94 10** (33 40)	2 016** (14 74)	0 402** (22 94)	19 90** (6 76)	1.23 (23)
200	8 05* (-12 22)	93 68** (32.81)	2.024** (15 20)	0 401** (22.63)	19 82** (6 33)	1 23 (23)
CONTROL	9 17	70.54	1.757	0.327	18 64	1 00
S Ed	0 0397	0.6107	0 0098	0.0020	0.1199	-
CD at 5%	0 0827	1.2740	0.0204	0 0042	0 2502	-
CD at 1%	0 1128	1.7375	0 0278	0 0057	0 3412	-

*Significant at 5%

**Significant at 1%

Value in parentheses are per cent increase (+)/decrease (-) over control values.

Fecundity, hatchability, larval duration, rate of development, viability, etc., are some of the important characters to evaluate the impact of any chemical or physical agents in animal test systems[16]. These serve as good indicators of various somatic effects caused by a chemical in the test substrate[17]. The parameters analysed in the present studies herald the impact of the spirulina on the economic characters of silkworm (Table 1). It produced no significant effect on the shell-ratio and silk-index of the silkworm.

Larval Duration :

The highest reduction in larval duration (-12.22%) was found in 200 ppm followed by –12.10% in 150 ppm and –11.02% in 100 ppm compared to the control. Similar stimulatory effect on larval period reduction in silkworm was reported due to ascorbic acid[18], thyroxine hormone[19] and potassium iodide at lower concentrations[20]. Reduced larval duration is attributed to the effect of oral administration of spirulina on acceleration of growth and development through orientation of physiological activity and thereby reduced the larval period. Digestibility of nutrients in phytophagous insects is comparatively lower than the herbivores. The inability of insects to digest the fiber portion of the diet and hence succulent mulberry leaves with less fiber and higher moisture and nutritive contents may enhance the quantity of silk produced by the silkworms, the reproductive potential of the adult and also reduce the larval period[21]. Hence, it is suggested that mulberry breeding requires qualitative improvement and particularly moisture and nutritive contents of the leaves and agronomic practices should aim at producing quality leaves as varieties, climate and agronomic practices influence the chemical composition of mulberry varieties[22].

Pupation rate :

The pupation rate was significantly increased by 33.40% in 150 ppm followed by 32.8% in 200 ppm and 11.33% in 100 ppm over control treatment ($P<0.01$). Considerable improvement in pupation rate presumed to be the stimulatory effect of dietary supplementation of spirulina. Such stimulatory effect on pupation rate due to idophor contents supplementation to silkworm, *B mori,* was also observed[23]. In silkworm breeding, selection for pupation plays a vital role, it increases the expected value and it ensures the progeny performance at a given probability and is as great as possible. Hence, it is suggested the silkworm breeder can effectively utilize the spirulina as dietary supplement to selected silkworm breeds for obtaining higher pupation rate for higher production of silkworm seed.

Cocoon characters

The cocoon and shell weights are characters which portray the productivity. The productivity depends on Darwinian fitness and viability. Further, viability is an adoptive trait of an individual, the increase of which qualities to survive itself and perpetuate in a given environment. The trait is also vulnerable to the changes in the internal and external factors. The cocoon weight of silkworm is the most important character, which is directly related with the production of silk. In the present investigation the highest improvement in cocoon weight (15.20%) was found in 200 ppm followed by 14.74% in 150 ppm 9.51% in 100 ppm, whereas the highest

improvement in shell weight (22.94%) was found in 150 ppm followed by 22.63% in 200 ppm and 20.49% in 100 ppm compared to the control. The highest shell percentage (20.48%) was found in 100 ppm followed by 19.90% in 150 ppm and 19.82% in 200 ppm compared to the control (Table 1). Enhanced cocoon characters have been observed in silkworm, *B. mori* L. due to vitamin enrichment [24-26]

It can be concluded that the oral administration of mulberry leaf supplemented with 100-150 ppm of spirulina as a feed to the silkworm, *B. mori* L. found to be effective in enhancing the larval and cocoon characters.

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